Development and Validation of a Novel Eco-friendly green Stability indicating RP-HPLC-PDA Method for the Simultaneous Quantification of Remogliflozin etabonate and Metformin HCl in Pharmaceutical Dosage Forms

Neha S. Mochi[1], Rajesh K. Patel[2]*

[1]Research Scholar, Gujarat Technological University, Ahmedabad, Gujarat, India.
[2]Associate Professor, Graduated School of pharmacy, Gandhinagar, Gujarat, India.
***Corresponding author: Dr. Rajesh K. Patel**
Department of Pharmaceutical Quality Assurance, Graduated School of Pharmacy, Gandhinagar, Gujarat. **E-mail:** raj07181@gmail.com

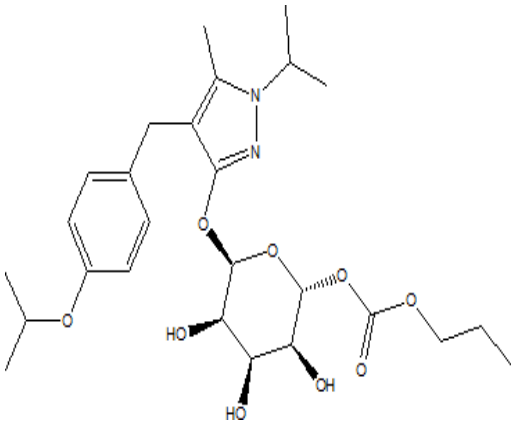
Abstract
The project sought to establish and test an environmentally friendly stability indicating RP-HPLC method for parallel quantification of Remogliflozin etabonate and Metformin HCl in their pharmaceutical combination dosage forms according to ICH guidelines. The analysis utilized Phenomenex C18 (250 mm × 4.6 mm, 5 µ) as the chromatographic column with a 45:55% v/v ratio of 0.02 M phosphate buffer (pH 3.5 adjusted with 1% OPA) and acetonitrile as the mobile phase and it operated at 1.0 mL/min through a PDA detector which found both medications and their degradant products had excellent peak separation at 245nm. The analytical method measured Metformin HCl retention time at 2.686 min along with Remogliflozin etabonate retention time at 5.842 min. The results confirmed that Remogliflozin etabonate exhibited linear behavior within 10-50 µg/mL along with Metformin HCl showing linear behavior from 50-150 µg/mL. A % recovery between 99-100 was achieved during the analysis. The precision analyses executed on all measurements produced results with %RSD values less than 2%. Analyses indicated that LOD equaled 0.11 µg/mL and LOQ stood at 0.35 µg/mL for Remogliflozin etabonate together with 0.24 µg/mL and 0.74 µg/mL values for Metformin HCl. Research findings during forced degradation assessments revealed that Remogliflozin etabonate undergoes high degradation levels when subjected to alkali solutions. Our proposed method scored excellent results in greenness analysis through AGREE and GAPI assessments. The established method demonstrated specificity together with precision as well as affordability and environmental friendliness making it ideal for standard quality assessment and stability tests to preserve the medicine's safety and effectiveness.

Introduction:

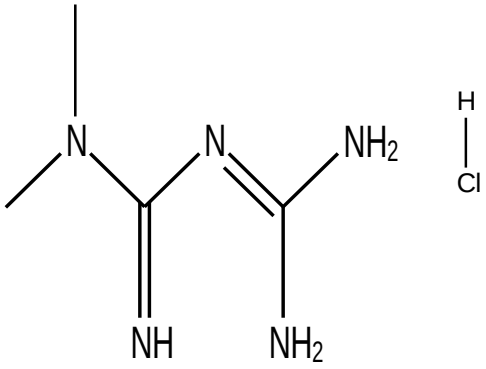
This orally-dosed Remogliflozin Etabonate serves as a Remogliflozin precursor to act as a renal sodium-glucose co-transporter subtype 2 inhibitor with antihyperglycemic effects which controls kidney reabsorption through activated benzylic acid releasing sites. The transport mechanism of glucose out of the blood occurs in urine due to the blocking action of this

transporter [1]. The drug substance functions orally as a Remogliflozin precursor which inhibits subtype 2 (SGLT2) renal sodium-glucose co-transporter inhibitors to control kidney reabsorption through activated benzylic acid releasing sites and displays antihyperglycemic properties. Urine excretes blood glucose because the transporter is blocked[1]. Taking Remogliflozin Etabonate by mouth works as a Remogliflozin opener to shut down subtype 2 (SGLT2) renal sodium-glucose co-transporter inhibitors that possess antihyperglycemic qualities to regulate kidney reabsorption from activated benzylic acid release sites. This chemical agent eliminates blood glucose by blocking the transporter [1]. The release of blood sugar to urine results from blocking this transporter despite the elimination process [1]. The chemical composition of this substance has been identified as 4 - [3 - amino - 4 - (2, 4, 5 - trifluorophenyl) butanoyl] - 3 - [(2-methylpropan-2-yl) oxymethyl] piperazin - 2-one; 2, 3 - dihydroxybutanedioic acid ethyl [(2R,3S,4S,5R,6S) - 3, 4, 5 - trihydroxy - 6 - {[5 – methyl – 1 - (propan – 2 - yl) – 4 - {[4 - (propan - 2yloxy) phenyl] methyl}-1H – pyrazol – 3 - yl] oxy} oxan – 2 - yl] methyl carbonate [6].

The medical compound Metformin hydrochloride functions as a primary type 2 diabetes mellitus treatment which patients take orally. This pharmaceutical substance contains 1,1-Dimethylbiguanide hydrochloride that forms the core structure of the compound. Pharmacologists select Metformin HCl as primary diabetes treatment because of its proven effectiveness combined with a secure profile and its weight-neutral properties. Metformin's working principles differ from most forms of oral antidiabetic drugs currently available. The therapeutic action of metformin consists of lowering glucose production during gluconeogenesis and reducing glucose absorption and making the body more sensitive to insulin while boosting peripheral glucose intake.[2-4,7].



Structure of Remogliflozin etabonate



Structure of Metformin HCl

People with diabetes who need better glycaemic control beyond Metformin HCl therapy can use combination tablets containing Remogliflozin etabonate and Metformin HCl as an effective control option. Different analytical methods such as UV spectrophotometry, RP-HPLC, and LC-MS/MS have been established for the assessment of either Remogliflozin etabonate or Metformin HCl alone or in combination in pharmaceutical tablets.[5-11] Individual RP-HPLC methods and two stability-indicating RP-HPLC methods currently exist for dual drug analysis. The assessment confirmed the development of an innovative RP-HPLC technology which provides both a quick and precise determination and exact measurement for Remogliflozin etabonate along with Metformin HCl within tablet formulations.

MATERIALS AND METHODS

Instruments

The Agilent 1260 Infinity II system with PDA detector successfully performed chromotographic separation of Remogliflozin etabonate alongside Metformin HCl. The HPLC system regulation and chromatographic data processing used the setup combination of CDS lab software together with the system configuration. The separation of Remogliflozin etabonate and Metformin HCl takes place effectively with the phenomenex C18 (250 mm x 4.6 mm) column featuring 5 µm particle size. The proposed work utilized an ultrasonic bath

sonicator from Labman as well as digital pH meters from Eutech Instruments alongside a UV-visible spectrophotometer from Shimadzu 1800.

Reagents and Chemicals

Remogliflozin etabonate were supplied by Glenmark pharmaceuticals Limited in Gujarat and Metformin HCl by Spensus Pharmaceuticals Ltd. in Gujarat. HPLC grade Water methanol was also provided by Finar Chemicals Pvt. Ltd., Gujarat, together with Acetonitrile. Potassium dihydrogen phosphate and trimethylamine were purchased from Rankem Chemicals Limited, Gujarat and the other chemicals such as sodium hydroxide, hydrochloric acid and hydrogen peroxide with orthophosphoric acid were purchased from Rankem Chemicals Limited.

Chromatographic conditions

Separation of Remogliloflozin etabonate and Metformin HCl was obtained using a Phenomenex C18 column (250 x 4.6 mm, 5 µm particle size). Study was done by mobile phase comprising of 0.02M phosphate buffer (pH 3.5, adjusted with 1 % OPA), 45 % acetonitrile, 55 % acetonitrile and the flow rate was 1 mL/min. The injection volume is 10 µL with UV detection at 245 nm.

Preparation of Buffer

The solution was prepared by mixing 1.36 g of potassium dihydrogen orthophosphate with 800 mL of water and 2 mL of triethylamine then being dissolved in water. Ortho phosphoric acid was then used to adjust the pH to 3.5. It was filtered into Whatman filter paper, degassed and then through Millipore nylon filter (0.45 µm). The sonication was thus finally performed for 10 minutes.

Preparation of mobile phase

A mixture of 45% v/v acetonitrile, 0.02M phosphate buffer (pH 3.5, buffer adjusted with 1% OPA) was prepared. At pH 3.5, OPA was used to adjust pH. Degassed for 10 minutes in a

sonicator and filtered through a 0.45 µm Millipore nylon filter, the solution was then prepared.

Preparation of diluents:

The drugs were dissolved in a mixture of ACN and water (50: 50 % v/v).

Preparation of standard stock solution

Twenty milligram of Remogliflozin etabonate and one hundred and fifty milligram of Metformin HCl were transferred into 10 mL volumetric flask containing a diluent mixture of acetonitrile and water (50:50% v/v) and the volume made up to 10 mL. It was made up to 100 mL in clean, dry glass vial. The standard solution was prepared by dissolving Remogliflozin etabonate to 300 µg/mL and Metformin HCl to 1500 µg/mL. The sample was passed through a 0.45 µm Millipore nylon filter which was degassed with a sonicator and dual ampouled. Remogliflozin etabonate and Metformin HCl stock solutions were further diluted with the diluent to obtain different concentrations of Remogliflozin etabonate and Metformin HCl.

Preparation of Sample Solution

Twenty tablets were weighed and the powdered transferred into a 10 ml volumetric flask. The amount of powder was adjusted to obtain 10 mg Remogliflozin etabonate and 50 mg Metformin HCl. For example, the solution was sonicated with 6 mL of diluent for 25 minutes. The diluent was adjusted to the volume after sonication and filtered. One hundred ten microliters of the filtered solution was transferred to a 10 mL volumetric flask, then fill the flask to the 10 mL mark with diluent. Evogliptin (30 µg/mL) and Metformin HCl tartrate (150 µg/mL) solutions filtered appropriately and properly diluted were prepared.

Forced degradation study

Stability profile of the drugs and potential degradation products can be determined through forced degradation studies. Such studies were normally carried out in acidic, thermal, photolysis and oxidative and basic conditions [13-15]. Standard stock solutions of the drug are

commonly prepared in suitable diluent for use in these studies. And the percentage of degradation degradation products were shown in Table 2 and analyzed.

Acid degradation

1 mL of 0.1 N HCl was added to 1 mL of the standard stock solution of Remogliflozin etabonate and Metformin HCl to 1 mL. It was stirred and refluxed at room temperature for about 1 hour. The contents were then allowed to reflux for a period of time, which were then cooled to the ambient temperature. Neutralization cooling is required to prevent thermal problem during the neutralization process. To neutralize the acidic solution 10 mL of 0.1N NaOH was added. Neutralization prevents the remaining acid from further acid degrading, and stabilizes the solution.

Base degradation

A 1 mL of the standard stock solution to Remogliflozin etabonate and Metformin HCl were taken to 1 mL. This mixture was refluxed put at room temperature for 10 minutes. The reflux period was maintained for a certain time followed by cooling to ambient temperature. There is a danger of sudden reaction in the neutralization process; thus, this needs to be cooled and 1 mL of 0.005N HCl was added to the acidic solution to neutralize it. Neutralization reduces further the degradation as the alkali is neutralized and the solution is stabilized.

Oxidative degradation

To the standard stock solution 1 mL of Remogliflozin etabonate and Metformin HCl, 1 mL of 3% H2O2 was added. The oxidizing agent was well mixed with the solution to achieve uniform distribution. After 24 hours, enough time allowed to complete the oxidative reaction, the mixture was stored at room temperature (RT). Following 24 hours, the final volume was brought to suitable diluent.

Thermal degradation

Determine the equivalence in the amount of Remogliflozin etabonate and Metformin HCl as

10 mg. Weigh out the needed amounts of powders and put them in a Petri dish and place them in an oven at 80°C for 4 hours. To study the effect of heat on the drug stability, this reaction is heated at this elevated temperature to simulate thermal stress conditions. Remove the Petri dish from the oven after 4 hours and let the contents cool to room temperature. Place the thermally stressed powders into a 100 mL volumetric flask. Add a suitable diluent to adjust to 100 mL. Make sure to mix very well to achieve uniform solution.

Photo Degradation

Find the mass of mixed Remogliflozin etabonate & Metformin HCl (Remogliflozin etabonate: Metformin HCl = 1 : 1). Take the drug powders and place them in a Petri dish. Place the Petri dish in UV chamber for 7 days. After 7 days of exposure transfer powders to 100 mL volumetric flask. The volume is adjusted to 100 mL with a suitable diluent. This should be well mixed so to make a uniform solution. The diluent was used to dilute the degraded sample solutions (from forced degradation studies) to give a final concentration of 100 µg/mL of Remogliflozin etabonate and 100 ug/mL of active ingredient of Metformin HCl. First, 100 µg/mL of Remogliflozin etabonate and 100 µg/mL of Metformin HCl were prepared in a mixture before analysis. It guarantees that both drugs are simultaneously analyzed in the same chromatographic conditions. The HPLC system was injected with 10 µL aliquot of each solution. The chromatographic conditions mentioned above are under which the analysis was carried out.

Method validation

The ICH requirements for accuracy, precision, linearity, specificity, limit of detection, limit of quantitation, and robustness were all met throughout the method's validation. [16-17]

Specificity

To ensure the analytical method can uniquely identify and quantify Remogliflozin etabonate and Metformin HCl and distinguish the analytes from any potential impurities, excipients, or

degradation products. A blank solution (diluent only, without the drugs) was injected under the optimized chromatographic conditions. A drug solution containing both Remogliflozin etabonate and Metformin HCl (10 µL) was injected into the column. Specificity was further validated by injecting solutions from the forced degradation studies (acid, base, oxidative, thermal, and photolytic conditions).

Linearity

Standard solutions of drugs Remogliflozin etabonate and Metformin HCl existed in established concentration levels of 10-50 µg/mL and 50-150 µg/mL respectively. The HPLC system received the chromatographically optimized method injection of prepared solutions. Areas under the peaks were recorded throughout all tested concentration ranges. To relate drug concentration levels with peak area measurements a linear regression analysis used the least square method. Scientists created calibration curves to represent both Remogliflozin etabonate drug and Metformin HCl drug. The linear relationship received assessment from a correlation coefficient measurement known as R^2.

Precision

A precision research analyzed the repeatability features alongside intermediate precision features for the analytical method of Remogliflozin etabonate and Metformin HCl determination. Six different sample solutions at 100% target concentration were used to determine repeatability (30 µg/mL Remogliflozin etabonate and 150 µg/mL Metformin HCl). The examined solution concentrations of 10, 30, and 50 µg/mL Remogliflozin etabonate and 50, 100, and 150 µg/mL Metformin HCl based on the linearity range were employed for precision data acquisition. The peak areas served to compute mean values and RSD% numbers.

Accuracy

The standard addition method will help determine the accuracy of this analytical method

through examination of known Remogliflozin etabonate and Metformin HCl solution recoveries added to pre-analyzed solutions. The standard drugs received three addition concentrations at 50%, 100% and 150% of pre-analyzed solution amounts. The analysis of each drug amount addition level was performed three times to confirm method consistency.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ values were determined in the laboratory for the detection limits of Remogliflozin etabonate and Metformin HCl by using the developed method. Three repeated calibration curve measurements were performed to analyze both of the drugs. Based on the mean slope data, the standard deviation of Y intercept data points was used to calculate detection limit (LOD) and quantification limit (LOQ) using the following formulas: LOD = 3.3 × (SD/Slope) and LOQ = 10 × (SD/Slope). Slopes are the mean value calculated from these curve slopes, and (SD) represents the standard deviation from three measurements of the calibration curve Y intercept.

Robustness

Robustness testing in analytical method validation, which assesses the reliability of a method when subjected to small, deliberate variations in experimental parameters. Robustness was achieved by adjusting small parameters like pH (± 0.05), flow rate (± 0.1 mL/min), and mobile phase composition (± 2% v/v).

System suitability parameters

Critical chromatographic parameters such as retention time, theoretical plate count, capacity factor and asymmetry factor were checked through a system suitability test. Before proceeding with the validation runs, this test was being done to make sure that the systems were throwing performance within expectations.

RESULTS AND DISCUSSION

Method development

Optimized Chromatographic Conditions

A refinement process was applied to the chromatographic system for optimal separation of Remogliflozin etabonate and Metformin HCl while controlling excipient and degradation product interferences. The mobile phase coupling with the column together with the flow rate enables superior separation power and high sensitivity to determine both drugs precisely in tablet dosage format. Table 1 presents all details about the optimized chromatographic conditions.

Table 1: Optimized Chromatographic Conditions

Parameter	Details
Elution Type	Isocratic
Mobile Phase	Phosphate buffer: Acetonitrile (45: 55 % v /v), pH 3.5 (adjusted with OPA)
Column	Phenomenex C18 (250 mm × 4.6 mm, 5 µm particle size)
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	245 nm
Total Run Time	10 minutes

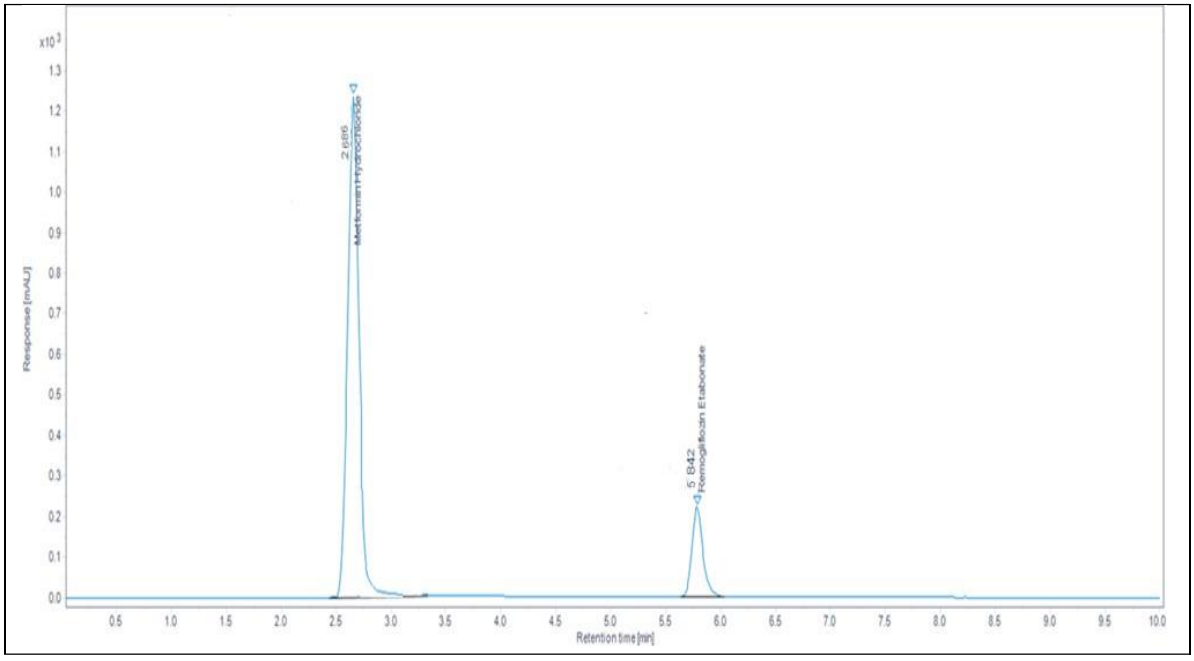


Figure 1: Chromatographic optimization for the separation of Remogliflozin etabonate and Metformin HCl

Forced degradation studies

Forced degradation studies were carried out in acid, base, thermal, photolytic and oxidative conditions; Significant decrease in the peak area for both Remogliflozin etabonate and Metformin HCl within 7 minutes. One additional peak observed at 3.642 min, indicating the formation of a degradation product (Fig. 2). Greater degradation of both drugs compared to other stress conditions. A notable reduction in the peak areas, suggesting high instability under basic conditions (Fig. 3). A significant decrease in peak areas for both drugs under oxidative stress. A small peroxide peak observed, merging with the Metformin peak around 2.404 min, potentially complicating the analysis (Fig. 4). A slight decrease in peak areas for both drugs under thermal conditions. Indicates moderate stability of the drugs when exposed to elevated temperatures (Fig. 5). A slight decrease in peak areas under photolytic conditions. Demonstrates relative stability of both drugs under light exposure (Fig. 6). The extent of degradation under each condition was summarized in Table 2.

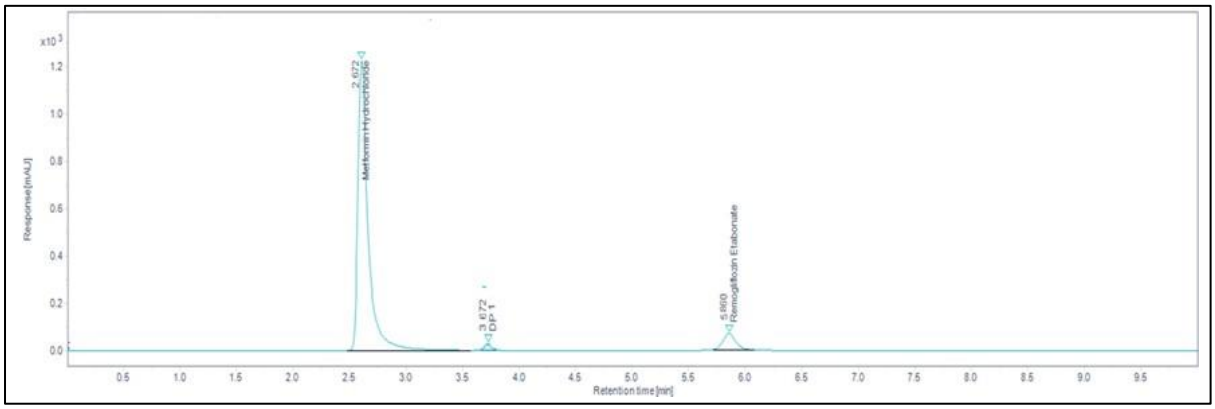


Figure 2: Chromatogram of acid degradation

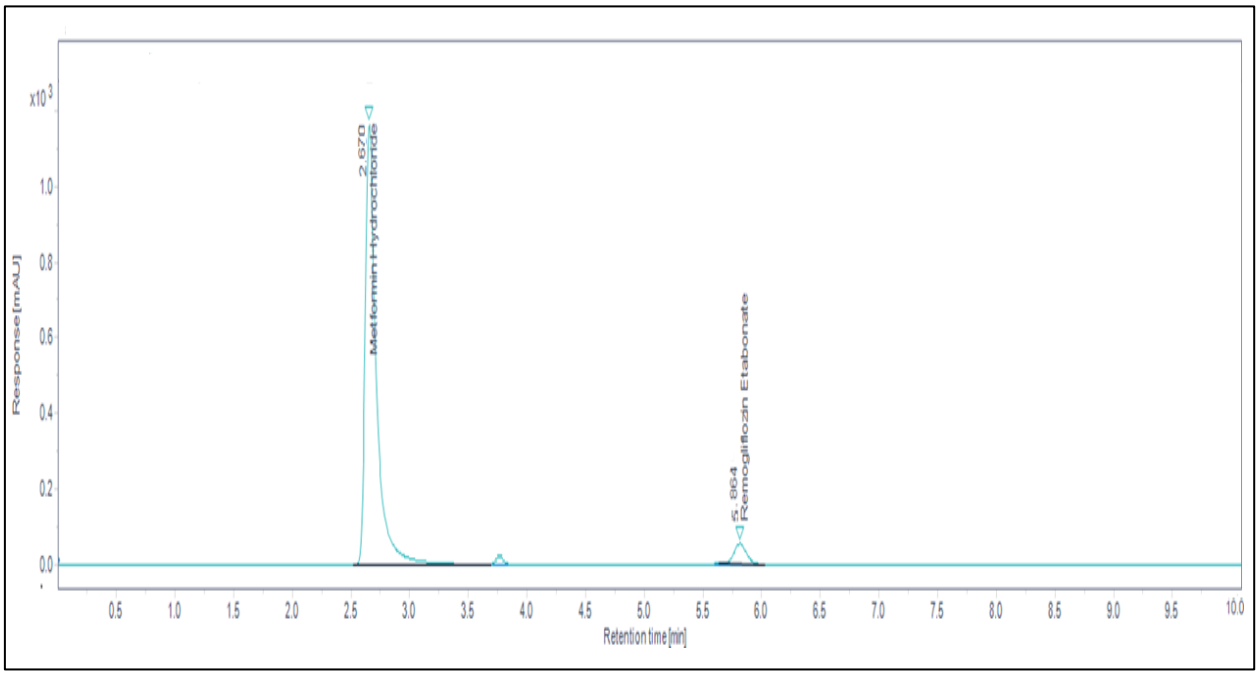


Figure 3: Chromatogram of base degradation

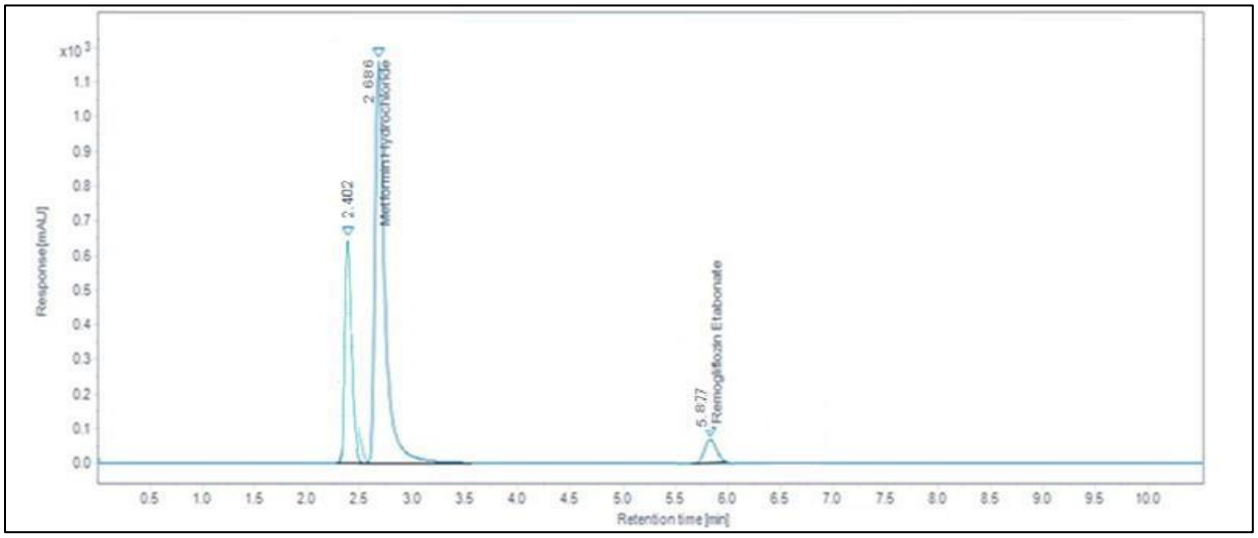


Figure 4: Chromatogram of oxidative degradation

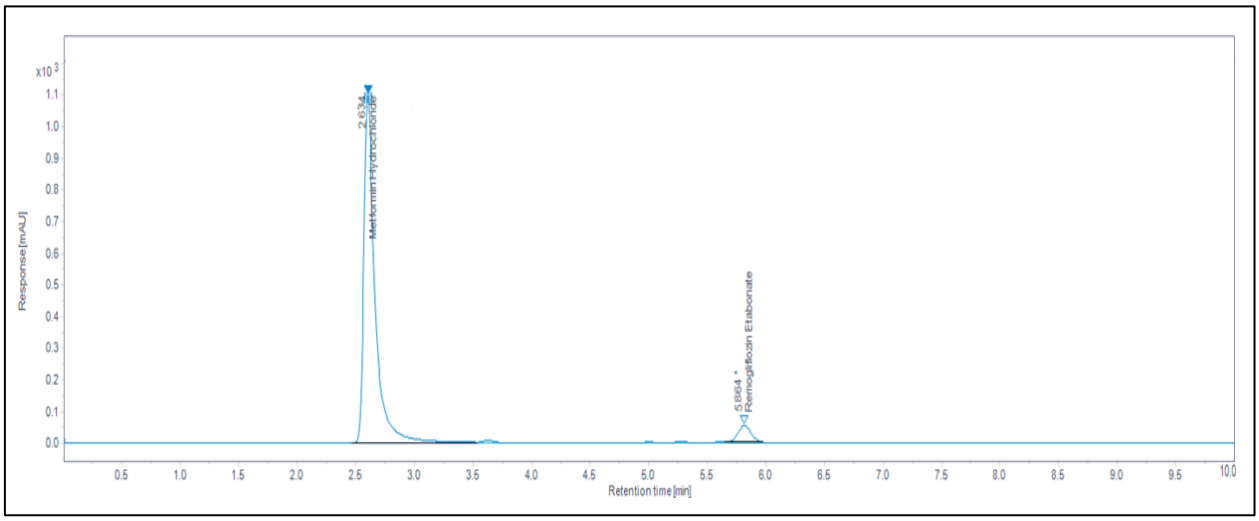


Figure 5: Chromatogram of thermal degradation

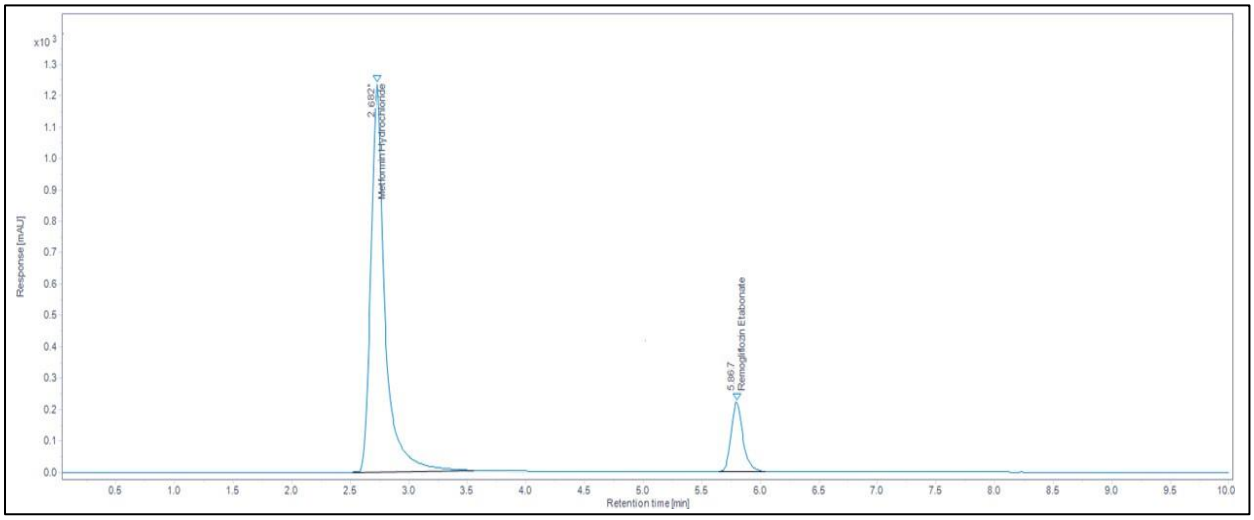


Figure 6: Chromatogram of photo degradation

Table 2: Degradation summary for Remogliflozin etabonate and Metformin HCl

Degradation conditions		Remogliflozin etabonate		Metformin HCl	
		% Degradation	Rt (min)	% Degradation	Rt (min)
Acid	0.1 N HCl (RT/ 1 hr)	19.16	5.860	0.83	2.672
Base	0.005 N NaOH (RT/ 10 min)	29.04	5.864	0.05	2.670

Thermal	80°C/ 4 hrs	10.09	5.866	1.22	2.681
Oxidation	3% v/v H_2O_2	4.72	5.864	1.42	2.634
Photo	UV light	1.59	5.867	1.05	2.682

Method validation

Specificity

The analytical specification tests the method's ability to detect and measure both Remogliflozin etabonate and Metformin HCl among all formulation ingredients and stress-degradation products formed by excipients and potential impurity compounds. Analysis of the blank solution and standard solution alongside sample solution was done through chromatographic measurement. The excipients showed no peaks that blended with either Remogliflozin etabonate or Metformin HCl peaks during the analysis. The experimental studies conducted under different conditions displayed separation between all degradation products and Remogliflozin etabonate and Metformin HCl peaks. The method displays specificity and readiness for stability analysis because the peaks of Remogliflozin etabonate, Metformin HCl and all their degradation products exhibit clear separation.

Linearity

The specified concentration ranges from 10 to 50 µg/mL for Remogliflozin etabonate and 50 to 150 µg/mL for Metformin HCl will be analyzed to determine the analytical method's linear response. Statistical parameters of drug calibration curves demonstrate the linear response relationship through determination of slope and intercept as well as the correlation coefficient. Table 3 and 4 together with Figures 7, 8, and 9 display the acquired results.

Table 3: Linearity data for Remogliflozin etabonate and Metformin HCl

Remogliflozin etabonate	Metformin HCl

Concentration (µg/mL)	Area (mAU) Mean±SD (n=3)	% RSD	Concentration (µg/mL)	Area (mAU) Mean±SD (n=3)	% RSD
10	179.70 ± 0.469	0.28	50	2651.05 ± 3.601	0.14
20	337.71 ± 1.478	0.44	100	5391.59 ± 8.303	0.15
30	510.67 ± 2.065	0.40	150	8061.02 ± 4.577	0.06
40	673.85 ± 2.017	0.30	200	10924.03 ± 10.386	0.10
50	849.31 ± 3.085	0.36	250	13271.99 ± 7.289	0.05

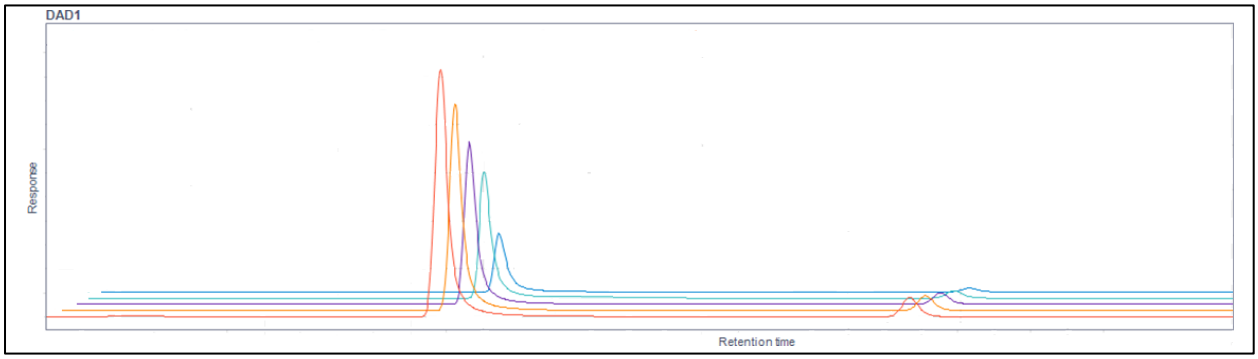


Figure 7: Overlain chromatogram of Remogliflozin etabonate and Metformin HCl

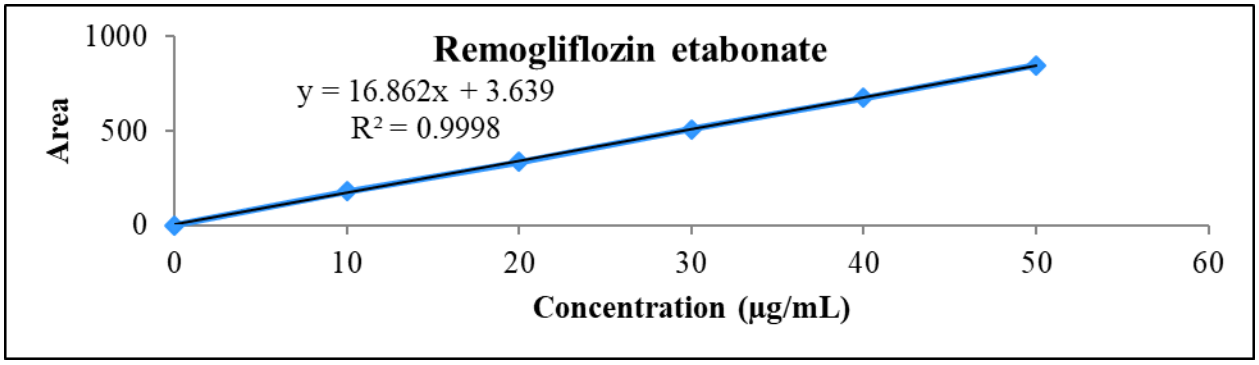


Figure 8: Calibration curve of Remogliflozin etabonate

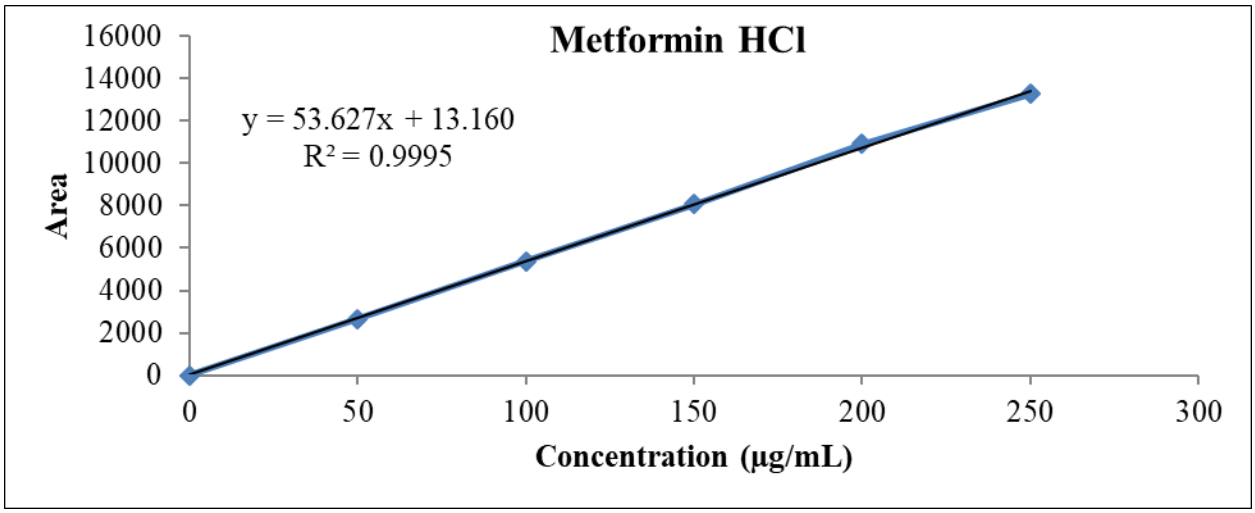


Figure 9: Calibration curve of Metformin HCl

Table 4: Calibration curve analysis using linear regression data

Parameters	Remogliflozin etabonate	Metformin HCl
Linearity range	10 - 50 µg/mL	50 - 150 µg/mL
Slope	16.862	53.627
Intercept	3.639	13.160
Correlation coefficient(r^2)	0.9998	0.9995

Precision

Six replicate preparations of drugs at 100% target concentration received full reporting within Table 5. The analysis of intermediate precision occurred for Remogliflozin etabonate at concentrations of 10, 30, and 50 µg/mL together with Metformin HCl at levels of 50, 100, and 150 µg/mL. Results indicated that ICH Q2(R1) guidelines establish 2% as the maximum allowed value for precision %RSD. The %RSD values obtained during Repeatability and intermediate precision research for Remogliflozin etabonate and Metformin HCl remained under the accepted limits. Table 5 alongside Table 6 displays the obtained results.

Table 5: Repeatability data for Remogliflozin etabonate and Metformin HCl

Drug	Conc. (µg/mL)	Mean Peak Area (n=6) ±SD,% RSD	Amount found ±SD,%RSD	%Amount found ±SD,% RSD
REMO	30	510.31 ± 2.647,0.519	30.13 ± 0.156, 0.520	100.44 ± 0.321,0.520
MET	150	8046.35 ± 13.32, 0.166	150.98 ± 0.251, 0.16	100.65 ± 0.167,0.165

Table 6: Intermediate precision data for Remogliflozin etabonate and Metformin HCl

Drug	Concentration (µg/mL)	Intraday Precision	Inter day Precision
		Mean±S.D (n=3), %RSD	Mean±S.D (n=3), %RSD
Remogliflozin etabonate	10	178.33 ± 1.29, 0.72	181.50 ± 1.91 , 1.05
	30	505.75 ± 4.63 , 0.91	510.34 ± 5.84 , 1.14
	50	840.65 ± 8.58 , 1.02	860.60±11.38 , 1.32
Metformin HCl	50	2654.74 ±±3.55 , 0.13	2655.31±4.34 , 0.16
	150	8073.69 ± 11.54 , 0.14	8074.39±15.99 , 0.19
	250	13294.40 ± 23.67 , 0.17	13300±27.56 , 0.20

Accuracy

The research evaluations using standard addition took place at three different sample levels ranging from 50% to 100% and 150% of the wanted concentrations. The drug recovery values obtained for both compounds ranged within 99% to 100% and satisfied ICH Q2 (R1) guideline requirements. The tables include recovery data with mean percentage findings and percentage relative standard deviations for both Remogliflozin etabonate and Metformin HCl.

Table 7: Accuracy data for Remogliflozin etabonate

SR. NO.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Total amount found (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery ± S.D, %RSD

							(n=3)
1	50 %	30	15	44.94	14.92	99.48	100.13 ± 0.598 , 0.597
2		30	15	45.12	15.10	100.65	
3		30	15	45.06	15.04	100.26	
4	100 %	30	30	59.92	29.90	99.67	100.0 ± 0.299 , 0.299
5		30	30	60.04	30.02	100.06	
6		30	30	60.10	30.08	100.26	
7	150 %	30	45	75.49	45.47	101.04	100.61 ± 0.458 , 0.456
8		30	45	75.08	45.06	100.13	
9		30	45	75.31	45.29	100.65	

Table 8 Accuracy data for Metformin HCl

SR. NO.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Total amount found (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery ± S.D, %RSD (n=3)
1	50 %	150	75	225.12	75.10	100.14	100.1 ± 0.150 , 0.151
2		150	75	225.19	75.18	100.24	
3		150	75	224.97	74.95	99.94	
4	100 %	150	150	300.30	150.28	100.19	100.17 ± 0.075 , 0.076
5		150	150	300.15	150.13	100.09	
6		150	150	300.37	150.35	100.24	
7	150 %	150	225	375.70	225.68	100.30	100.20 ± 0.099 , 0.100
8		150	225	375.47	225.46	100.20	

9		150	225	375.25	225.23	100.10	

Limit of detection (LOD) and limit of quantitation (LOQ)

You would obtain the SD of intercepts by calculating and determining the standard deviation from five calibration curve intercepts. Results are shown in Table 9.

Table 9: LOD and LOQ data for Remogliflozin etabonate and Metformin HCl

Parameters	Remogliflozin etabonate	Metformin HCl
LOD (µg/mL)	0.11	0.24
LOQ (µg/mL)	0.35	1.79

Robustness

The examination of robustness involved changing pH value and flow rate and mobile phase composition for analyzing Remogliflozin etabonate at 30 µg/mL and Metformin HCl at 150 µg/mL. All experimental results produced acceptable outcomes. The study revealed that RSD did not exceed 2.0%. Tables 10 and 11 present the obtained outcomes.

Table 10: Robustness data for Remogliflozin etabonate

Drug name	Parameters	Optimized condition	Used condition	Mean Area (mAU) (n=3)	Rt (min) (n=3)	Plate count (n=3)	Tf (n=3)	% Assay (n=3)
µg/mL)	Flow rate (±0.1mL/min	1 mL/min	0.9 mL/min	516.46	5.805	14602	1.034	101.66
			1.0 mL/min	510.31	5.834	14572	1.022	100.45
			1.1	507.06	5.895	14633	1.026	99.81

			mL/min					
			Avg	511.28	5.830	14602.33	1.027	100.64
			SD	4.77	0.023	30.501	0.006	0.939
			%RSD	0.93	0.385	0.209	0.595	0.933
	Mobile phase (± 2%v/v)	**45:55 % v/v**	**43:57% v/v**	506.00	5.869	14635	1.019	99.60
			45:55% v/v	510.31	5.834	14572	1.022	100.45
			47:53% v/v	514.44	5.854	14644	1.016	101.26
			Avg	510.25	5.819	14617.000	1.019	100.44
			SD	4.22	0.059	39.230	0.003	0.830
			%RSD	0.83	0.996	0.268	0.294	0.827
	pH (± 0.05)	**3.5**	**3.45**	511.48	5.883	14554	1.032	100.68
			3.5	510.31	5.834	14572	1.022	100.45
			3.55	514.18	5.842	14645	1.026	101.21
			Avg	511.99	5.820	14590.333	1.027	100.78
			SD	1.99	0.032	48.191	0.005	0.391
			%RSD	0.39	0.541	0.330	0.490	0.388

Table 11: Robustness data for Metformin HCl

Drug name	**Parameters**	**Optimized condition**	**Used condition**	**Mean Area (mAU) (n=3)**	**Rt (min) (n=3)**	**Plate count (n=3)**	**Tf (n=3)**	**% Assay (n=3)**

Metformin HCl (300 µg/mL)	**Flow rate (±0.1mL/min)**	**1 mL/min**	**0.9 mL/min**	8062.33	2.659	4425	1.415	100.85
			1.0 mL/min	8046.35	2.6728	4437	1.432	100.66
			1.1 mL/min	8041.76	2.692	4467	1.448	100.60
			Avg	8050.15	2.6746	4443	1.431	100.70
			SD	10.80	0.016	21.63	0.016	0.131
			%RSD	0.13	0.619	0.486	1.15	0.130
	Mobile phase (± 2%v/v)	**45:55 % v/v**	**43:57% v/v**	8042.43	2.69	4508	1.446	100.40
			45:55% v/v	8046.35	2.672	4437	1.432	100.66
			47:53% v/v	8033.07	2.667	4473	1.415	100.79
			Avg	8040.62	2.676333	4472.66	1.431	100.62
			SD	6.82	0.012097	35.501173369	0.015	0.198
			%RSD	0.08	0.451993	0.793736183	1.084	0.196
	pH (± 0.05)	**3.5**	**3.45**	8048.09	2.702	4469	1.449	101.03
			3.5	8046.35	2.672	4437	1.432	100.66
			3.55	8075.74	2.655	4429	1.421	100.68

			Avg	8056.73	2.676	4445.000	1.434	100.79
			SD	16.49	0.024	21.166	0.014	0.207
			%RSD	0.20	0.889	0.476	0.984	0.205

Table 12: Summary of validation parameters

Parameters		**Remogliflozin etabonate**	**Metformin HCl**
Linearity range (n=3)		10-50 µg/mL	50-250 µg/mL
y = mx + c		y = 16.8627x + 3.6397	y = 53.6276x + 13.1603
Correlation coefficient(r^2)		0.9998	0.9995
Precision (%RSD)	0.51	0.16	0.11
	0.72-1.02	0.13-0.17	0.11-0.17
	1.05-1.32	0.16-0.20	0.19-0.22
Accuracy (%Recovery)		100.00-100.61	100.10-100.20
LOD(µg/mL)		0.11	0.24
LOQ(µg/mL)		0.35	0.74
Specificity		Specific	Specific
Robustness		Robust	Robust

System suitability parameters

The optimal analytical conditions allowed Remogliflozin etabonate to elute at 5.834 minutes while Metformin HCl needed 2.677 minutes. The peak symmetries of both test compounds

remained under 1.5 thus demonstrating satisfactory peak distribution according to USP standards (peak symmetry or tailing factor should ideally fall below 2.0). The obtained plate numbers exceeded 2000 for both compounds due to the efficient operation of the column. Six standard injections showed %RSD values of less than 2 percent for both analyte area measurements thereby proving precision. A summary table presents the data which can be found in Table 12.

Table 13: System suitability parameters data for Remogliflozin etabonate and Metformin HCl

Parameters	Remogliflozin etabonate			Metformin HCl		
(n=6)	Observation	SD	%RSD	Observation	SD	%RSD
Retention Time (min)	5.834	0.016	0.28	2.677	0.005	0.18
Theoretical Plates	4435	9.486	0.21	14572	16.04	0.11
Tailing factor	1.02	0.0011	0.11	1.43	0.0021	0.15
Resolution	17.63	0.019	0.10	-	-	-

Analysis of marketed formulation

The analysis of Remogliflozin etabonate along with Metformin HCl in the Remo M 500 tablets was conducted through duplicate assessments of sample and standard drugs at identical concentrations to their dosage proportions in the formulation. A table with the results appears in Table 13.

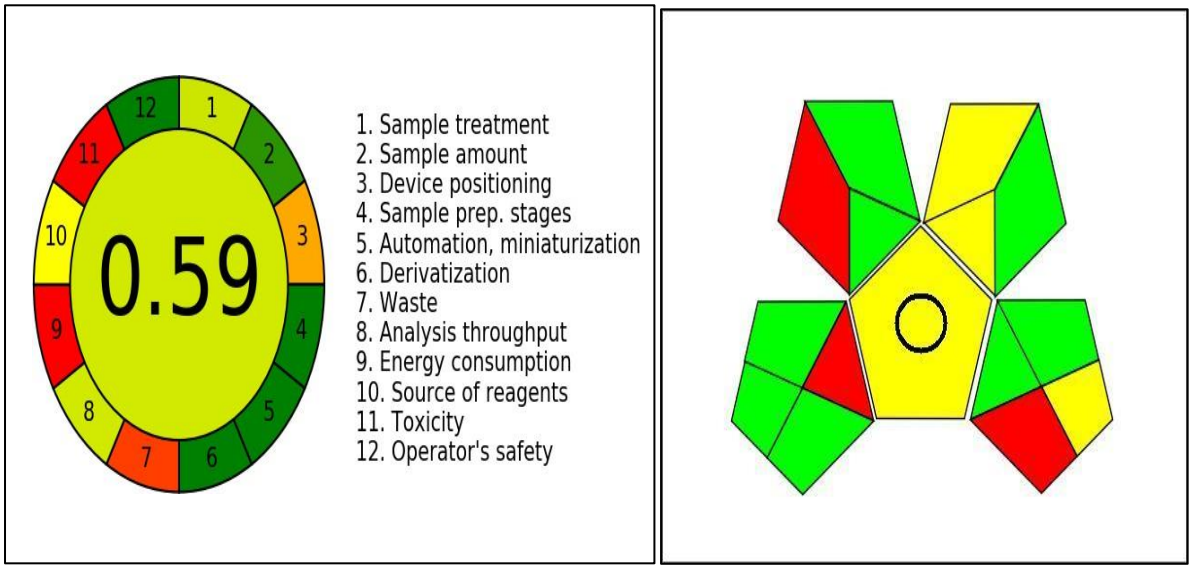
Table 14: Analysis of marketed formulation

Label Claim (mg/tablet)		Assay (Content in mg)		% Assay (Mean ± S.D)	
Remogliflozin etabonate	Metformin HCl	Remogliflozin etabonate	Metformin HCl	Remogliflozin etabonate	Metformin HCl

100	500	100.12	500.08	100.12 ± 0.687	100.01 ± 0.046

Greenness assessment of developed method

A greenness evaluation of the developed method assesses sustainability and environmental footprint through assessment of solvent consumption alongside energy usage and waste production and ecological impact. The method followed a green chemistry approach which decreased environmental harm while maintaining performance criteria by using renewable resources together with non-hazardous materials. The evaluation used the AGREE and GAPI tools that follow the twelve principles of Green Analytical Chemistry (GAC). The AGREE assessment yielded poor ratings in energy consumption (principle 9) and toxic reagent usage (principle 11) but secured high ratings for principles 2, 4, 5, 6 and 12. The method demonstrated a low environmental impact according to the measured AGREE score which reached 0.59 (light green). Three red zones were identified within the GAPI pictogram related to physico-chemical properties, non-greener solvent utilization and insufficient waste processing as depicted in Fig. 10.



A **B**

Figure 10: (A) AGREE results (B) GAPI results for developed method

CONCLUSION

Scientists established a new stability-indicating HPLC method for evaluating Remogliflozin etabonate and Metformin hydrochloride (HCl) in their combined drug formulation following International Conference on Harmonisation (ICH) guidelines. The established method displayed linear detection of Remogliflozin etabonate concentrations between 10 and 50 µg/mL along with Metformin HCl concentrations spanning from 50 to 150 µg/mL. Both analytes showed excellent linear performance (r^2 = 0.999) throughout their investigated ranges. Recovery tests confirmed that percentage recoveries of Remogliflozin etabonate and Metformin HCl stayed within 90-100% range as per the accuracy acceptance criteria. The inspection of experimental precision through % RSD showed values of less than 2% which verified both method stability and reproducibility. This analytical method presents simple operation procedures together with excellent selectivity and precision and accuracy and robustness characteristics for regular pharmaceutical dosage form testing of both drugs. The stability-indicating features of the validated method were confirmed by separate detection of degradation substances from the target compounds which ensures monitoring drug stability within pharmaceutical formulations and storage environments. The green characteristics of the developed method underwent evaluation using the renowned AGREE and GAPI software tools. The developed method achieves functionality as a pharmaceutical analysis method that follows green chemistry principles by being cost-efficient and operator-safe and environmentally friendly. The research offers a validated quality control assessment that is dependable and environmentally sustainable for investigating the stability of Remogliflozin etabonate and Metformin HCl fused dosage forms. This method presents itself as a suitable

technology for widespread adoption during pharmaceutical formulations' quality control assessments and stability tests for active pharmaceutical ingredients.

REFERENCES

1. Rang, H. P., Dale, M. M., Pitter, J. M., & Flower, R. J. (2007). *Pharmacology* (6th ed.). Elsevier.
2. Goyal, R. K. (2009). *Elements of Pharmacology* (19th ed.). B. S. Shah Prakashan. Indian Pharmacopoeia Commission. (2010).
3. *The Indian Pharmacopoeia* (6th ed., Vol. II). Indian Pharmacopoeia Commission.
4. British Pharmacopoeia Commission. (2013). *British Pharmacopoeia* (Vol. II). Her Majesty's Stationery Office.
5. Beckett, A. H., & Stellate, J. B. (2005). *Practical Pharmaceutical Chemistry* (Part II, 1st ed.). CBS Publishers and Distributors.
6. DrugBank. (2016, October). *Remogliflozin etabonate.* https://go.drugbank.com/drugs/DB12935
7. DrugBank. (2005, June). *Metformin HCl.* https://www.drugbank.ca/salts/DBSALT000114
8. Madhukar, A., Prince, A., Vijay, K. R., Sanjeeva, Y., Jagadeeshwar, K., & Raghupratap, D. (2011). Simple and sensitive analytical method development and validation of metformin hydrochloride by RP-HPLC. *International Journal of Pharmacy and Pharmaceutical Sciences, 3*, 117-120.
9. D'souza, S., Krishna, M., Sushmitha, G. S., & Vasantharaju, S. G. (2018). Stability indicating assay method development and validation to simultaneously estimate metformin hydrochloride and canagliflozin by RP-HPLC. *Current Trends in Biotechnology and Pharmacy, 12*, 334-342.

10. Chengalva, P., Parameswari, A. S., & Aruna, G. (2018). Development and validation of RP-HPLC method for metformin hydrochloride and nateglinide in bulk and combined dosage form. *International Journal of Research in Pharmaceutical Sciences, 9*, 267-271.
11. Bhavyasri, K., Surekha, T., & Sumakanth, M. (2020). A novel method development and validation of dapagliflozin and metformin hydrochloride using simultaneous equation method by UV–Visible spectroscopy in bulk and combined pharmaceutical formulation including forced degradation studies. *Journal of Pharmaceutical Sciences and Research, 12*(8), 1100-1105.
12. Ayoub, B. M., & Mowaka, S. (2017). LC–MS/MS determination of empagliflozin and metformin. *Journal of Chromatographic Science, 55*(7), 742-747.
13. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2005). *ICH Q1A (R2): Stability testing of new drug substances and products*. ICH.
14. Singh, S., & Bakshi, M. (2000). Conduct of stress tests to determine inherent stability of drugs. *Pharmaceutical Technology Online*, *24*(2), 1-14.
15. Singh, S., & Bakshi, M. (2002). Development of validated stability-indicating assay methods—Critical review. *Journal of Pharmaceutical and Biomedical Analysis, 28*(6), 1011–1040.
16. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2005). *ICH Q2 (R1): Validation of analytical procedures, text and methodology*. ICH.
17. United States Pharmacopeial Convention. (2000). *United States Pharmacopeia/National Formulary* (24th ed., p. 2149). Rockville, MD: U.S. Pharmacopeial Convention.