RP-HPLC Method Development and Validation for the Estimation of Remoaglyflozin Etabonate

N.Y. Bairagi[1*], D.M. Sonawane[2], S.S. Aher[3], R.S.Bachhav[4]
[1,2]*Department of Quality Assurance, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India*
[3]*Department of Pharmaceutical Chemistry, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India*
[4]*Department of Pharmacology, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India*

Abstract: Remogliflozin etabonate, a prodrug of gliflozin, is a medication used to treat type 2 diabetes and non-alcoholic steatohepatitis. It inhibits the kidney's SGLT2 protein, reducing glucose reabsorption and increasing urine glucose excretion. This drug is absorbed orally and is converted into its active form, Remogliflozin, which helps lower blood glucose levels. A study aimed to develop a simple, accurate, and suitable reversed-phase high-performance liquid chromatography (RP-HPLC) method for determining Remogliflozin Etabonate in bulk drugs and pharmaceutical formulations. The developed method uses an isocratic program with a mobile phase of Methanol and water at a flow rate of 1.0 ml/min. The analysis was performed on an HPLC system equipped with a UV-visible detector, utilizing Openlab EZ-Chrome Software and a Kromasil C18 column. The results were validated for linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The method offers advantages such as reproducibility, rapid analysis, simple sample preparation, enhanced selectivity and sensitivity. The method is well-suited for routine analysis of Remogliflozin Etabonate in bulk drugs and pharmaceutical dosage forms in the pharmaceutical industry.

Keywords: Developed method, Remogliflozin etabonate, Analyte, Detection, Qualification

INTRODUCTION

Remogliflozin etabonate, also known as (5-methyl-4-[4-(1-methylethoxy) benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D glucopyranoside, is a member of the gliflozin family. It is a prodrug of gliflozin, which is mostly prescribed for type 2 diabetes and non-alcoholic steatohepatitis. Transport proteins, glucose re-inclusion in the kidney, and sodium-glucose reduction are all facilitated by RMZ. A substance that is soluble in methanol, ethanol, and DMSO is an antidiabetic drug that is produced by either relative or total insulin action and/or excretion. A survey of the literature shows that there aren't many methods available for using HPLC to determine the etabonate of remogliflozin. This class of medications acts by blocking the kidney's SGLT2 protein, which is in charge of reabsorbing glucose into the bloodstream. [1]

Remogliflozin Etabonate works by inhibiting a specific protein, which reduces glucose reabsorption and increases its excretion through urine, helping to lower blood sugar levels. It is a prodrug, meaning it is converted in the body to its active form, Remogliflozin, which provides the therapeutic effect. This drug is usually taken orally and is mainly used to treat type 2 diabetes mellitus, either alone or in combination with other antidiabetic medications like metformin.[2] After oral administration, Remogliflozin Etabonate is rapidly absorbed and hydrolyzed into the active form, Remogliflozin. The active form then inhibits SGLT2, leading to an increase in urinary glucose excretion. This mechanism helps reduce blood glucose levels in patients with type 2 diabetes.[3]
Remogliflozin Etabonate is quickly absorbed and converted into Remogliflozin, the active form, upon oral administration. Then, the active form inhibits SGLT2, increasing the amount of glucose excreted in the urine. This process assists individuals with type 2 diabetes in lowering their blood glucose levels.[4]

MATERIALS AND METHODS:[5-7]

Materials: Intas Pharmaceuticals generously provided a pure sample of Remogliflozin Etabonate as a gift. All chemicals and solvents used were sourced from Merck Pharmaceutical in Mumbai and were of HPLC grade.
Instruments: Chromatographic measurements were conducted with an Agilent Model No. 1260 Infinity II HPLC Binary Gradient System and a Double beam UV-visible spectrophotometer manufactured by JASCO UV 550.
Chemicals and Reagents: Acetonitrile and methanol, two chemicals of HPLC quality, were purchased from Merck Specialties Private Limited in Mumbai.

Preparation of standard stock solution for Chromatographic development [8]:
Remogliflozin etabonate Standard Stock Solution was prepared for that 25 mL volumetric flask was cleaned and dried, and 25 mg of remogliflozin etabonate was transferred into it. About 15 mL of methanol was then added to completely dissolve the material and bring the volume up to the required level. (PPM of 1000) used mobile phase to further dilute 2.5 ml of the stock solution to 25 mL. (One hundred parts per million). Analytical wavelength selection for the development of HPLC methods. The wavelength of maximal absorption found in the spectrophotometric study, 228 nm, was chosen as the analytical wavelength for the investigation.

Preparation of Mobile Phase:
Prepare the mobile phase by Adjust the methanol: water ratio (75:25) to prepare the mobile phase.
Chromatographic Conditions:
Standard solution: Remogliflozin etabonate100 PPM
Detector: U.V. Detector
Column: Kromasil C18
Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 35°C
Injection Volume: 20 µl
Wavelength: 228 nm

Method Validation:[9-14]

1. Linearity and Range

An analytical process is said to be linear if it can produce test findings that show a clear, consistent relationship between the amount or concentration of the analyte in the sample throughout a certain range The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 5 levels of Linearity were performed from 10% to 150% of working concentration

2. Limit Of Detection (LOD) and Limit of Quantitation (LOQ)

The lowest concentration of an analyte in a sample that can be identified but may not always be quantified as an exact number is known as the detection limit of a particular analytical technique. Quantitation limit: The lowest concentration of analyte in a sample that can be quantitatively measured with appropriate precision and accuracy is the quantitation limit of a single analytical process.
$LOD = 3{:}3 \times \sigma / S$
$LOQ = 10 \times \sigma / s$
Were,
σ = residual standard deviation of a regression line
S = Slope of regression line

3. Precision

The degree of agreement between several measurements taken from numerous samplings of the same homogenous test conducted under the specified conditions is expressed as the precision of an analytical method.

4. Accuracy

The degree of agreement between the value found and the value that is recognized as either a conventional true value or an acceptable reference value is expressed by the analytical procedure's accuracy. Between fifty percent and one hundred fifty percent of working concentration will be used for accuracy. Three copies of each accuracy level's solution were made. computed the mean percent recovery for each level and overall recovery as well as the percentage RSD for each level and the overall recovery. The recovery percentage for each sample, the recovery means for each level, and the recovery total were calculated. Furthermore, percentage RSD for each stage and percentage RSD for the overall recovery were calculated. The maximum value for the percentage RSD should be 2.0%.

5. Robustness

An analytical procedure's resilience to tiny, intentional changes in method parameters is measured by its robustness, which also indicates how reliable it is under typical operating conditions. The resilience of the approach was exhibited by deliberately adjusting the temperature, flow velocity, and detecting wavelength while estimating the tablet by ± 2°C, ± 0.1 ml, and ± 3 nm, respectively. The reproducible results obtained show the resilience of the method.

RESULTS AND DISCUSSION

Selection of analytical wavelength 1) Blank Methanol:
Blank Methanol

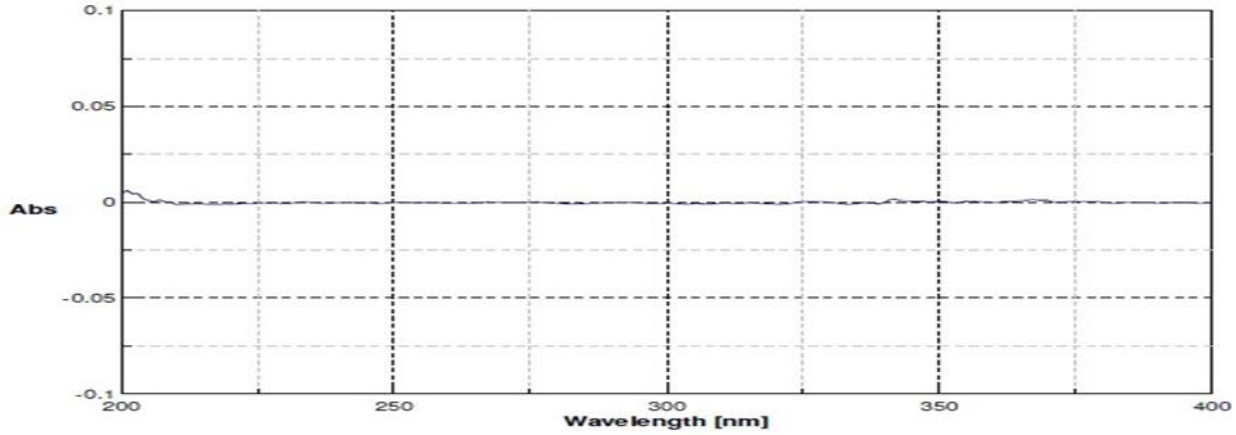

Fig No:1 UV spectrum of methanol as a blank

2) Remogliflozin etabonate STD solution: (20 PPM)

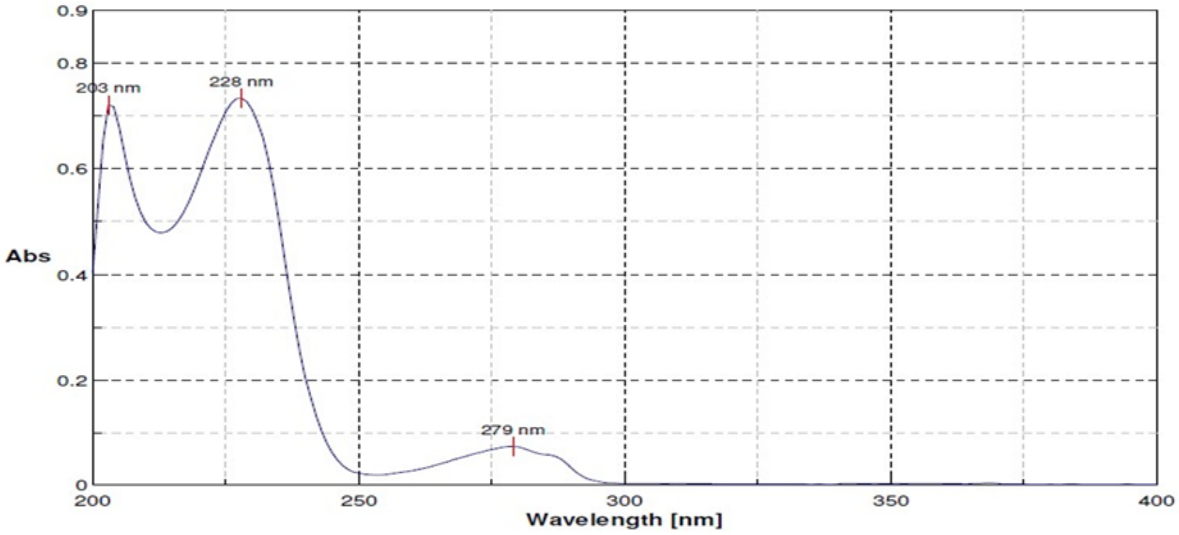

Fig. No. 2 UV spectrum of Remogliflozin etabonate

Development of HPLC method for Remogliflozin etabonate

A high-performance liquid chromatographic technique was created and approved to measure bulk Remogliflozin etabonate. The composition of the mobile phase is Methanol: Water (75-25% V/V). The obtained chromatogram indicates that 228 nm is the maximum wavelength at which the medication exhibits its highest response.

Chromatogram:

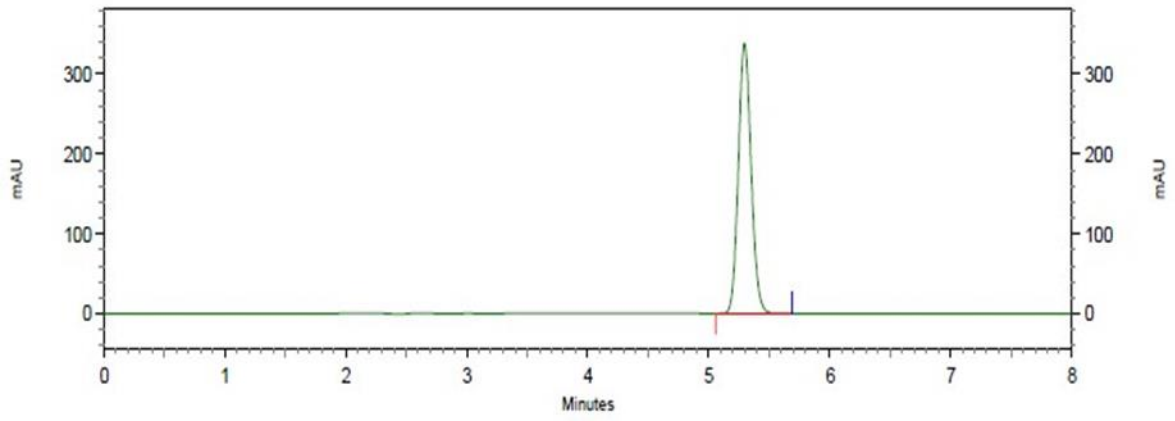

Fig No:3Typical chromatogram of Remogliflozin etabonate

Linearity

The drug was shown to be linear between 10 and 100 µg/ml in concentration. The calibration plot obtained was displayed in Figure, and the results are displayed in the Table below.

Table No.*1* : Data of calibration curve of Remogliflozin etabonate by HPLC method

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	2.00	936012	933625	0.227
		932904		
		931960		
50%	10.00	4639784	4639875	0.441
		4660359		
		4619482		
100%	20.00	9360489	9341636	0.234
		9346713		
		9317707		
125%	25.00	11629456	11568136	0.648
		11590345		
		11484607		
150%	30.00	14016237	14021729	0.112
		14009476		
		14039473		

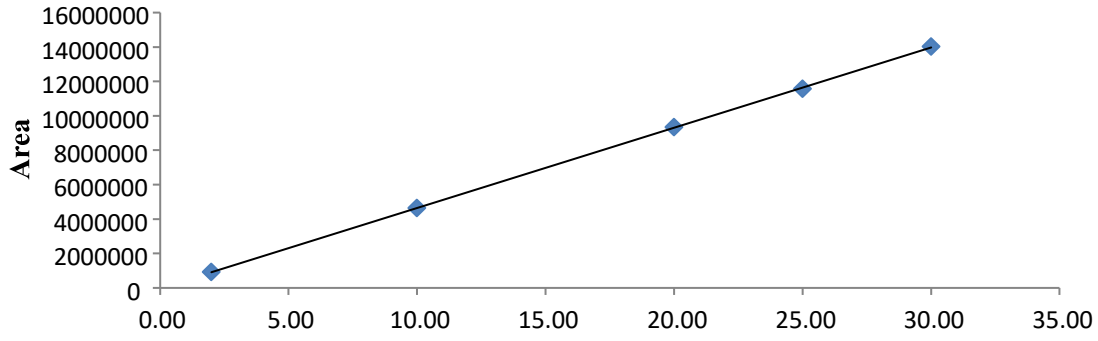

Fig. No. 4 Calibration curve of Remogliflozin etabonate

Table No:2 Data of linearity of Remogliflozin:

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	2.0 - 30.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99996	NLT 0.98
3	Intercept	-10229.07648	To be report
4	Slope	466162.6021	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Remogliflozin etabonate was:

Y = M X + C

Y = 466162.6021 x + -10229.07648 where, x = concentration of Analyte in µg/mL y = is area of peak.

M = Slope

C= Intercept

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 47516.81 (Residual standard deviation of a regression line)

s = 466162.6021 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 47516.81 / 466162.6021

LOD = 0.336 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 47516.81 / 466162.6021

LOQ = 1.019 µg/mL

Accuracy:

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Results of recovery study are shown in and statistical validation is shown in Table

Table No.3 : Result and statistical data of Accuracy of Remogliflozin etabonate

Level (50 %)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4669584	10.01	10.08	99.31	98.67	0.5607
	4589315	9.84	10.00	98.40		
	4623781	9.91	10.08	98.31		
100	9320485	19.98	20.16	99.11	99.29	0.4549
	9350146	20.04	20.08	99.80		
	9270756	19.87	20.08	98.95		
150	14109476	30.24	30.08	100.53	99.61	0.9719
	13838940	29.66	30.08	98.60		
	13957914	29.91	30.00	99.70		

Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2. Result of intraday and interday precision was shown in Table below.

Table No:4 Result of Intra- day and Inter- Day Precision for Remogliflozin etabonate test sample assay:

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	86.5	9306451	99.50
	Sample 2	86.2	9226014	98.98
	Sample 3	86.3	9291450	99.57
	Sample 4	86.3	9126780	97.80
	Sample 5	86.4	9248561	98.99
	Sample 6	86.5	9374560	100.23
	Mean			99.18

	STD DEV			0.8170
	% RSD			0.824
Intermediate precision (Inter-Day)	Sample 1	86.2	9210542	98.81
	Sample 2	86.6	9123095	97.42
	Sample 3	86.1	9376801	100.72
	Sample 4	86.3	9200458	98.59
	Sample 5	86.5	9210961	98.48
	Sample 6	86.4	9294721	99.49
	Mean			98.92
	STD DEV			1.1072
	% RSD			1.119
Repeatability Plus Inter-day	Mean			99.048
	STD DEV			0.9376
	% RSD			0.947

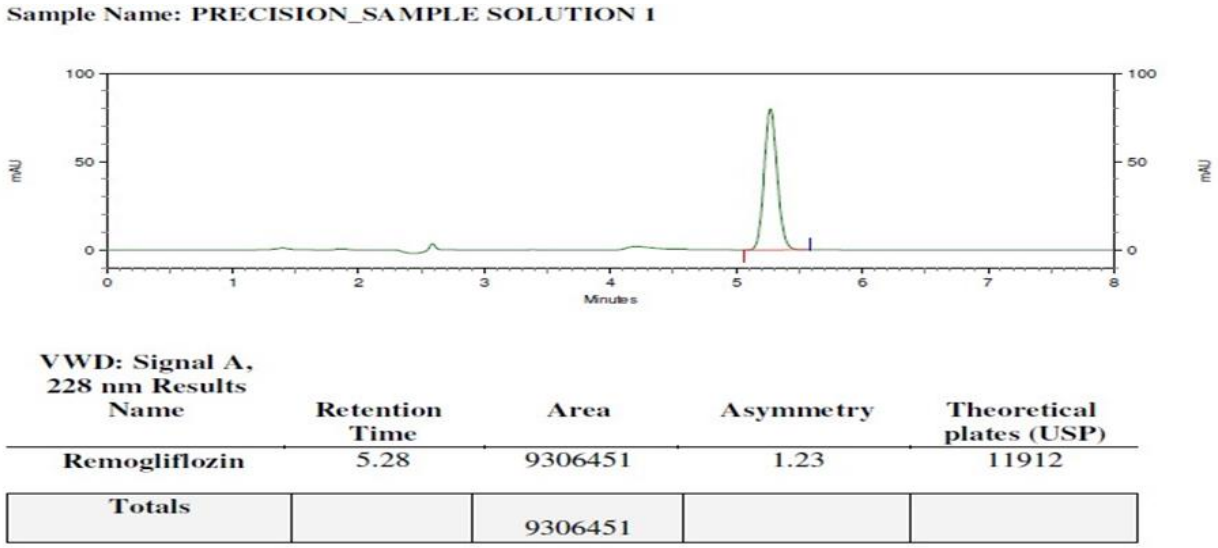

Fig no: 5 Typical chromatogram of Repeatability precision

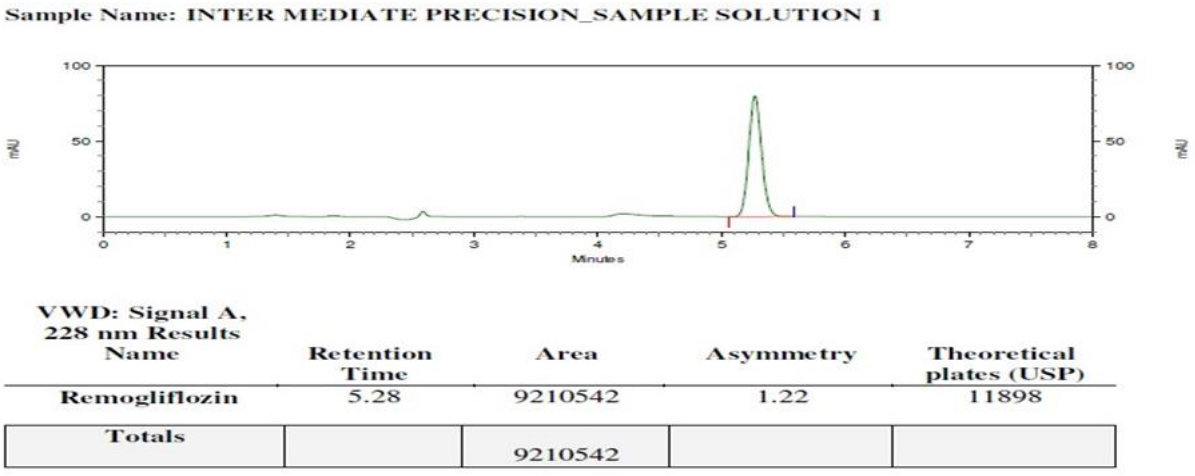

Fig No:6 Typical chromatogram of Inter-day precision

Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions. Results are shown in Table

Table No.7 : Data for Robustness study of Acebrophylline by HPLC method

Sr. No.	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	1026207	1028587	2451.4	0.23833
2		1	1028449			
3		1.1	1031104			
1	Change in Wavelength (nm)	273	1030728	1030045	1386.62	0.13462
2		275	1028449			
3		277	1030957			

Table No:8 Result of Robustness study

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 nm (231 nm)	5.28	9136081	1.20	11891
Wavelength by -3 nm (225 nm)	5.28	9067914	1.19	11826
Flow rate by +10% (1.1mL/min)	4.80	8529170	1.21	11146
Flow rate by -10% (0.9mL/min)	5.86	10418906	1.21	12205
Column oven temp by +2ºC (37 ºC)	5.29	9260479	1.24	11916
Column oven temp by -2ºC (33 ºC)	5.28	9308751	1.23	11870

SUMMARY

This study focused on developing a simple, accurate, precise, and suitable RP-HPLC method. A review of existing literature revealed that several methods have been previously reported for determining Remogliflozin Etabonate in bulk drugs or pharmaceutical dosage forms. Therefore, this study aimed to develop and validate a new, sensitive, and effective reversed-phase high-performance liquid chromatography (RP-HPLC) method for determining Remogliflozin Etabonate in bulk drugs and pharmaceutical formulations.

In the developed RP-HPLC method, the analyte was separated using an isocratic program with a mobile phase consisting of Methanol and water (75:25) at a flow rate of 1.0 ml/min. The analysis was performed on an HPLC system equipped with a UV-visible detector, utilizing Openlab EZ-Chrome Software and a Kromasil C18 column (250 mm x 4.6 mm, 5 µm). Detection was carried out at 228 nm.

The results of the analysis using this method were validated for linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The developed method offers several advantages, including reproducibility of results, rapid analysis, simple sample preparation, and enhanced selectivity and sensitivity. The regression coefficient (r^2) for each analyte was not less than 0.999, indicating good linearity. The percentage recovery was within the acceptable range for tablet dosage forms, and the %RSD was below 2%, demonstrating a high degree of precision.

Due to its robustness, reproducibility, and efficiency, the developed method is well-suited for routine analysis of Remogliflozin Etabonate in bulk drugs and pharmaceutical dosage forms in the pharmaceutical industry.

CONCLUSION

The assay for Remogliflozin Etabonate is conducted using a validated RP-HPLC method, ensuring accuracy, precision, linearity, robustness, ruggedness, system suitability, limit of detection, and limit of quantification. The method has been proven to be accurate, precise, linear, robust, and rugged, in line with ICH guidelines.

This summary provides a comprehensive overview of the development and validation of an analytical method for Remogliflozin Etabonate, which is essential for maintaining consistent quality in pharmaceutical products. The developed method was found to be simple, sensitive, accurate, and precise. It can be used for the routine analysis of Remogliflozin Etabonate without interference from the excipients used in the formulation.

REFERENCE

[1] Nauck, Michael. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Design, Development and Therapy. 2014, 8: 1335–80.

[2] Nashawi, Mouhamed, Sheikh, Omar, Battisha, Ayman, Ghali, Abdullah, Chilton, Robert. Neural tone and cardio-renal outcomes in patients with type 2 diabetes mellitus. a review of the literature with a focus on SGLT2 inhibitors. Heart Failure Reviews. May 2021, 26 (3): 643–652.

[3] Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya U, Shah DCN. Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug remogliflozin etabonate. Future Journal of Pharmaceutical Sciences, 2021, 7; 1-12.

[4] Tushar Bhatkar, Amol V. Badkhal, Nitin S. Bhajipale (2017). "Stability Indicating RPHPLC Method Development and Validation for the Estimation of Remogliflozin Etabonate in Bulk and Pharmaceutical Dosage Form." Int. J. Pharm. Res.vol. 9(3), pp.2366-2372

[5] Dave Vidhi, Paresh Patel, (2021). "Method Development and Validation of UV Spectrophotometric estimation of Remogliflozin Etabonate in bulk and its tablet dosage form." Res. J. Pharm. Technol. vol. 14(4), pp.2042-4.

[6] Beckett A.H, Analytical Chemistry and Practical Chemistry. 1996; 14th edi. –Vol-2.: 27595

[7] ICH, Q2R1, Text on validation of analytical Procedures International Conference on harmonization, Geneva, 1994 October,1-13

[8] Kokkeragadda Akhila, Dr. Srinivasa Rao N ; NEW VALIDATED METHOD FOR THE ESTIMATION OF REMOGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORM USING RP-HPLC; Journal Of Emerging Technologies And Innovative Research (JETIR) ISSN: 2349-5162 ESTD Year: 2014

[9] Patil, R., Deshmukh, T., Patil, V., Khandelwal, K. (2014). Review on Analytical Method Development and Validation. Research and Reviews: JPA, 3(3), July-September, 1-10.

[10] Skoog D.A, Holler F.J, Timothy A, Nieman N.W, Principle of instrumental Analysis. Eastern Press, Bangalore 2004; 5th edi.: 1-4,729-35

[11] Willard H.H, Merritt L.L, Dean J.A, Settle Jr. F.A, Instrumental Methods of analysis. CBS Publishers and distribution, Delhi, 2001; 7th edi.: 118-19

[12] Sushil D. Patil , Development and Validation of Simple UV-Spectrophotometric Method for the Determination of Empagliflozin, Asian Journal of Pharmaceutical Analysis 7(1):18 ,2017

[13] Gupta, V., Jain, A. D. K. J., N. S, G., & Guptan, K. (2012). Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2(4), 17-25.

[14] Pushpa Latha, E., & Sailaja, B. (2014). Bioanalytical method development and validation by HPLC: a review. Journal of Applied Pharmacy, 1, 1-9.