Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 2169-2181 | ISSN:2251-6727

Implementation of Quality by Design (QbD) approach to the analytical method development and validation for the estimation of Remogliflozin Etabonate in tablet dosage form by HPLC

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(**Received**:14 June 2024 **Revised**: 01 July 2024 **Accepted**: 18 August 2024)

KEYWORDS
QbD, Remogliflozin, RP-HPLC, Method development, Anova, Validation

ABSTRACT:

Analytical Quality by Design is a recent concept related to the development and validation of the analytical procedures (AQbD). This is done in analytical QbD by defining quality targets which enable the performance of the analytical procedure within the MODR. This is because AQbD considers Out Of Trend (OOT) and out of specification (OOS) results by the quality of the procedure. Therefore, the QbD approach to analytical development is preventative, systematic, and is based on risk which greatly assists in understanding how differences in CPPs impact on CQAs. Thus, DoE plays a crucial role in QbD, for executing the response surface analysis and the screening process, and finally for defining the area with several dimensions of the successful operating ranges of the CPPs, supposed as Design Space (DS).

The objectives of this paper are to describe the development and validation of a rapid, sensitive and specific RP-HPLC method for the determination of Remogliflozin etabonate in its bulk drug and tablet dosage forms. In this method Column with mobile phase Methanol: Water (90:10) was applied in the enhancement of the method. For Remogliflozin etabonate the retention time at the wavelength of 225 nm was 4.48 min to complete and meets the ICH guidelines in the aspects of accuracy, linearity, precision, robustness, LOD, and LOQ.

Introduction –

Remogliflozin Etabonate is an Anti-diabetic drug, chemically known as 5-Methyl-4-[4-(1-methylethoxy)benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl-6-O-(ethoxycarbonyl)-β-D Glucopyranoside. It inhibits the sodium-glucose transport proteins (SGLT), which are responsible for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine. The structural formula is shown in Fig 1.

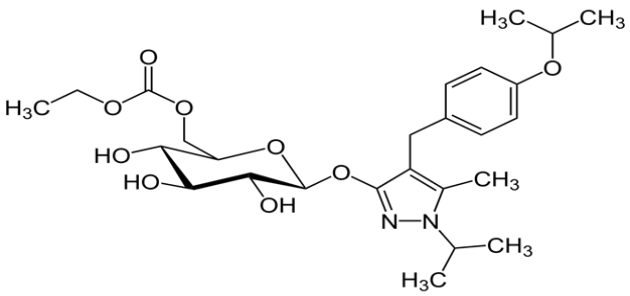

Fig 1. Structure of Remogliflozin etabonate

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Analytical Quality by Design-

AQbD is a concept that involves applying the principles of QbD in the development of analytical methods within a method operable design region (MODR) instead of conventional analytical methods. The cause is the implementation of the AQbD approach in method development that results in minimizing the incidence of OOT and OOS due to the effectiveness of the methodology within the designated zone. The FDA in 2011 linked ICH Q9 on risk management to analytical methods. Presently, AQbD is widely applied in the pharmaceutical industries as one of the risk management tools especially in method development. The application of QbD entails establishing a clear relationship between product attributes and process parameters through the definition and description of the product's design space which is a multi-parametric region. Some of the concepts that are similar to QbD in manufacturing processes can be associated with the creation of analytical methods. The implementation of AQbD starts with identification of the ATP that defines the intended use of the analytical procedure. It stresses the importance of the critical method parameters (CMPs) that are set and defined based on miscellaneous analysis and assessment of risk methodologies during the key phase of familiarise with the analytical system. The working intervals of the key elements of the method (CMPs).Within a multidimensional region that enables the achievement of the desired critical values method attributes (CMAs), the space is referred to as the design space (DS). The first aspect of QbD is to create ATP, which is similar to the Quality Target Product Profile (QTPP). In the second phase of Quality by Design (QbD), Critical Quality Attributes (CQAs) are identified, Risk assessment in analytical Quality by Design (AQbD) is the evaluation of the analyst's procedure, instrument parameters, analysis parameters, material attributes, sample preparation, and environmental conditions to determine the risk of variability in CQA. The role of the analyst is to define the design space of the method based on the DoE outcomes originated from the response modeling, and more specifically for the case of L16, higher augmentation of response surface design. This space is depicted in a 3D graph where contour plots of all the responses are overlay on one graph based on factors and their interaction. The area enclosed by the experimental boundary is referred to as the design space in which all CMA attributes have been satisfied, demonstrating the method's reliability. Creation of a control strategy involves the skills of the analyst and understanding of the Method Operable Design Region (MODR). All statistical data gathered during the MODR can be used to develop the method's control strategy. Experimental design can be defined as the use of statistical, mathematical and graphical techniques for the purpose of improving one or more responses subject to certain constraints whereby the response algorithms have to be stated appropriately and data is collected, arranged and analyzed.

Materials –

A sample of Remogliflozin Etabonate is gifted by Metrochem Pvt Ltd based in Hyderabad, India. REMO®-ZEN 100 mg tablet is manufactured by Glenmark Pharma Limited. For the method's development, HPLC grade chemicals were used they were purchased from Merck Hyderabad, India.

Equipments-

The chromatographic separation was done on a Cosmosil C18 column (265mm x 4.8 ID, 5µ) with a P-3020-M Reciprocating pump (40MPa), and UV detector with HPLC workstation software. For the spectroscopic analysis a double beam UV-visible spectrophotometer was used while a wenser high precision electronic balance was used for weighing in the study.

Chromatographic conditions –

Mobile Phase consists of Methanol the solvent system used was water in (90:10) ratio. The flow rate was 0.9 ml/min with the injection volume of 20µl. The UV Detection wavelength was 225 nm and temperature was set at ambient and separation was carried out. Stoichiometry of the reaction and preparation of reference standard solution. To make a 1000 parts per million of each of the drugs be prepared with a standard stock solution.

Selection of detection wavelength –

The UV-VIS scan used on the solution of Remogliflozin etabonate was in the range of 200-400nm. The selected wavelength for the analysis was 225 nm.

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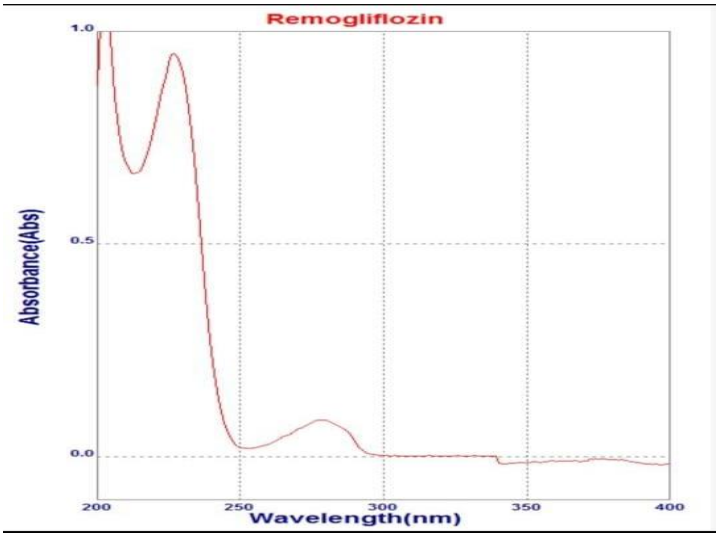

Fig 2. UV spectra of Remogliflozin

HPLC method development by QbD approach- Analytical target product profile, Retention time, theoretical plates, peak area, and asymmetry factor were the QTPP selected in order to optimize the chromatographic conditions of HPLC.

Critical quality attributes- The mobile phase composition of methanol to water, 90:10, retention time, and wavelength were identified.

Factorial design- The Box-Behnken experimental design was selected for the HPLC method development.

Design Space- The study type for the response surface, the Box-Behnken design, and the quadratic design model with 17 runs were used. The mobile phase composition retention time and wavelength were evaluated against the four responses retention time, area, theoretical plates, and asymmetry factor using the suggested Box Behnken experimental design. The result was then summarized.

Anova for Quadratic model

Response 1: Retention time

Source	Sum of squares	Df	Mean Square	F- value	P- value	
Model	227.102	9	25.2336	354.709	1.81E-08	Significant
A-Composition	204.546	1	204.546	2875.31	2.06E-10	
B- flow-rate	3.76065	1	3.76065	52.8637	0.00017	
C- wavelength	5.51E-05	1	5.51E-05	0.00077	0.97857	

Table 1. ANOVA for the Quadratic Model: Retention time

The Model F-value of 354.71 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, AÂ² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model

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Fit Statistics			
Std. Dev.	0.266718	RÂ²	0.997812
Mean	8.479941	Adjusted RÂ²	0.994999
C.V. %	3.145285	Predicted RÂ²	0.964993
		Adeq Precision	56.14023

Table 2. Fit statistics

The Predicted RÂ² of 0.9650 is in reasonable agreement with the Adjusted RÂ² of 0.9950; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 56.140 indicates an adequate signal. This model can be used to navigate the design space.

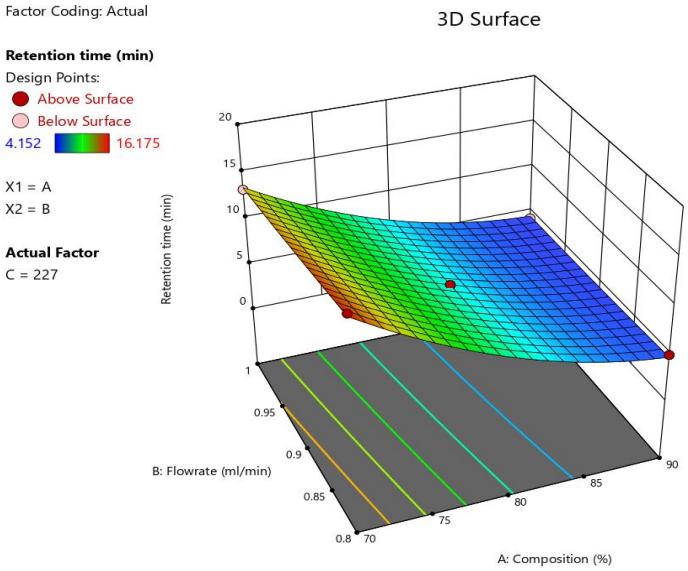

Fig 3. 3D surface response of retention time

Response 2: Area

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	3.64E+11	9	4.04E+10	22.2757	0.000239	Significant
A-Composition	6.29E+08	1	6.29E+08	0.346201	0.574757	
B-Flowrate	1.08E+11	1	1.08E+11	59.5749	0.000114	
C-Wavelength	5.09E+08	1	5.09E+08	0.280374	0.612827	

Table 3. ANOVA for the Quadratic Model: Area

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The Model F-value of 22.28 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, AÂ² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics			
Std. Dev.	42611.7242	RÂ²	0.96626202
Mean	1065434.18	Adjusted RÂ²	0.92288461
C.V. %	3.99947037	Predicted RÂ²	0.4601923
		Adeq Precision	14.7950418

Table 4. Fit Statistics

The Predicted RÂ² of 0.4602 is not as close to the Adjusted RÂ² of 0.9229 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.795 indicates an adequate signal. This model can be used to navigate the design space

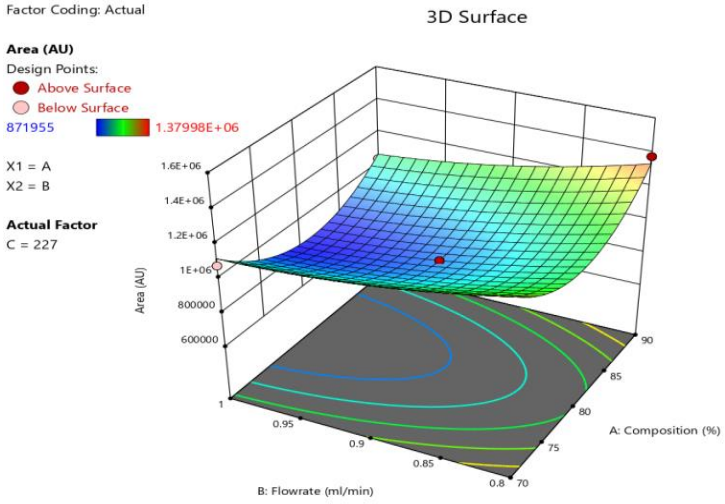

Fig 4. Response Surface Design 3D model for the Area

Response 3 : Theoretical plates

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	4008110	9	445345.6	13.86643	0.00111	significant

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A-Composition	455535.1	1	455535.1	14.18369	0.007018	
B-Flowrate	305762	1	305762	9.520307	0.017678	
C-Wavelength	162735.1	1	162735.1	5.066975	0.059111	

Table 5. ANOVA for the Quadratic Model: Theoretical plates

The Model F-value of 13.87 implies the model is significant. There is only a 0.11% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, BC, AÂ², CÂ² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics			
Std. Dev.	179.2117	**RÂ²**	0.946888
Mean	7750.588	**Adjusted RÂ²**	0.878602
C.V. %	2.312233	**Predicted RÂ²**	0.150214
		Adeq Precision	12.92388

Table 6. Fit Statistics

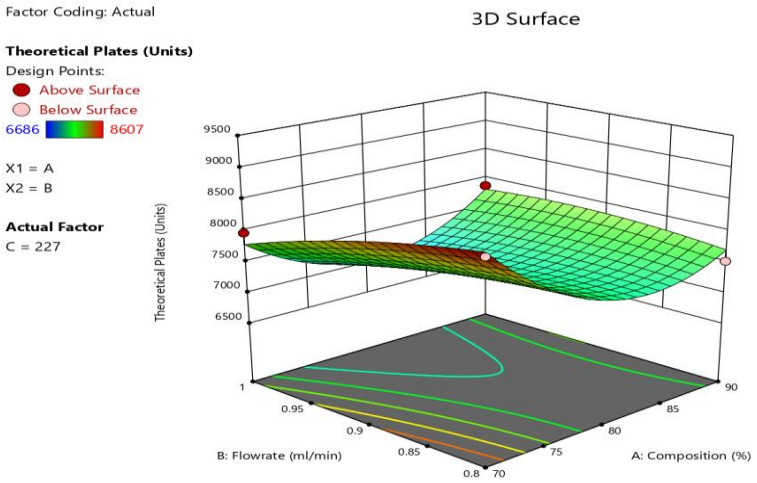

Fig 5. Response Surface Design 3D model for the Theoretical plates

Response 4: Asymmetry factor

Source	**Sum of**	**Df**	**Mean**	**F-value**	**p-value**	

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	Squares		Square			
Model	0.003799	9	0.000422	4.727059	0.02637	significant
A-Composition	0.000313	1	0.000313	3.5	0.103552	
B-Flowrate	5.00E-05	1	5.00E-05	0.56	0.478644	
C-Wavelength	1.25E-05	1	1.25E-05	0.14	0.719357	

Table 7. ANOVA for the Quadratic Model: Asymmetry Factor

The Model F-value of 4.73 implies the model is significant. There is only a 2.64% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case BC, AÂ², BÂ², CÂ² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics			
Std. Dev.	0.0094491	RÂ²	0.8587101
Mean	1.3152941	Adjusted RÂ²	0.6770517
C.V. %	0.718403	Predicted RÂ²	-1.260638
		Adeq Precision	8.4516123

Table 8. Fit statistics

A negative Predicted RÂ² implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.452 indicates an adequate signal. This model can be used to navigate the design space.

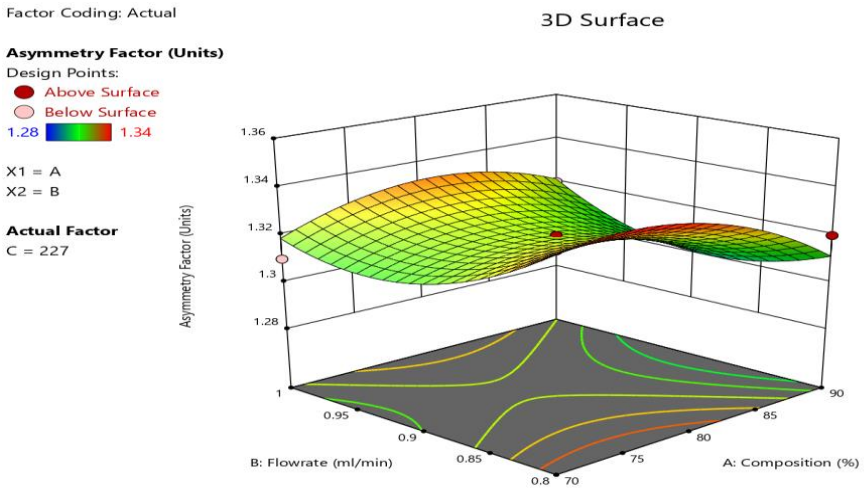

Fig 6. Response Surface Design 3D model for the Asymmetry factor

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Optimized condition obtained

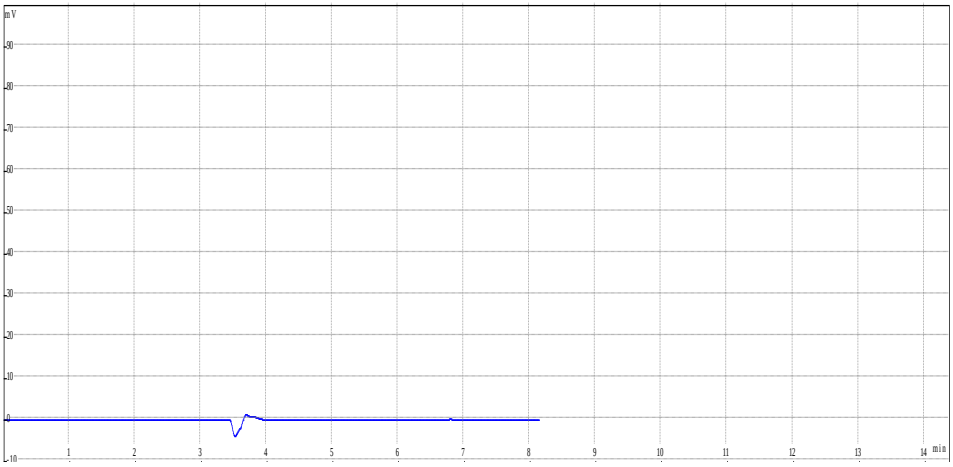
The data was collected by considering of all the responses in the different experimental conditions and the software used for this purpose was Design expert version 13.0.5.0. Retention times of the compounds of interest and the method of detection were established and the expected results are shown.

Run 14 Response	Predicted Mean	Predicted Median	CI for Observed
Retention Time	4.667	4.667	3.951
Area	1379964.03	1379964.03	1265668.76
Theoretical Plates	7879.275	7879.27	7398.58
Asymmetry Factor	1.2744	1.2744	1.24

Table 9. Point prediction and confirmation

The observed value for responses was determined by injection of the chromatogram with a particular composition of the mobile phase, flow rate and wavelength and comparing the results with the predicted ones to calculate % prediction error.

Method validation- This method was validated according to the ICH guidelines



Fig– 7. Blank trial of remogliflozin

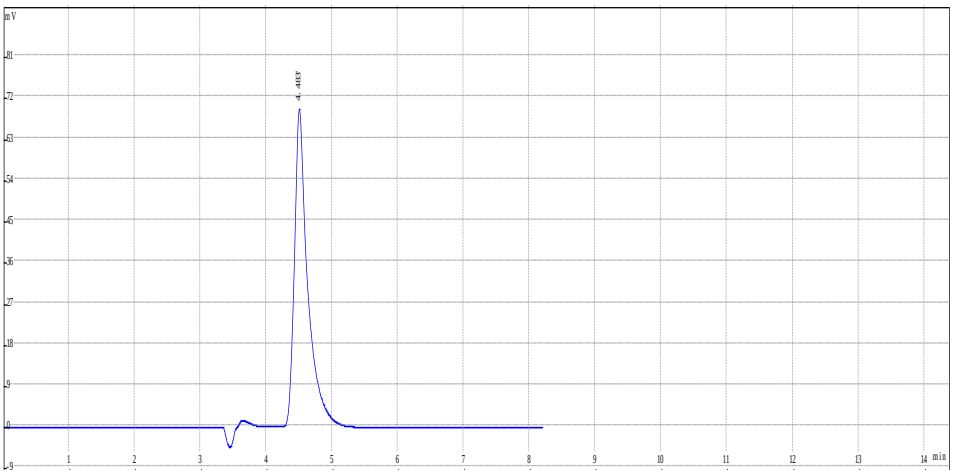


Fig -8. Optimized trial of remogliflozin

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System suitability

The appropriateness of the method was evaluated using six individual examinations of Remogliflozin. For standard solutions, the parameters such as retention time, efficiency of the column, asymmetry factor and theoretical plates were evaluated.

Linearity –

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.

Concentration(µg/mL)	Area
10	236322
20	455730
30	718550
40	959210
50	1208350

Table 10. Linearity data of Remogliflozin

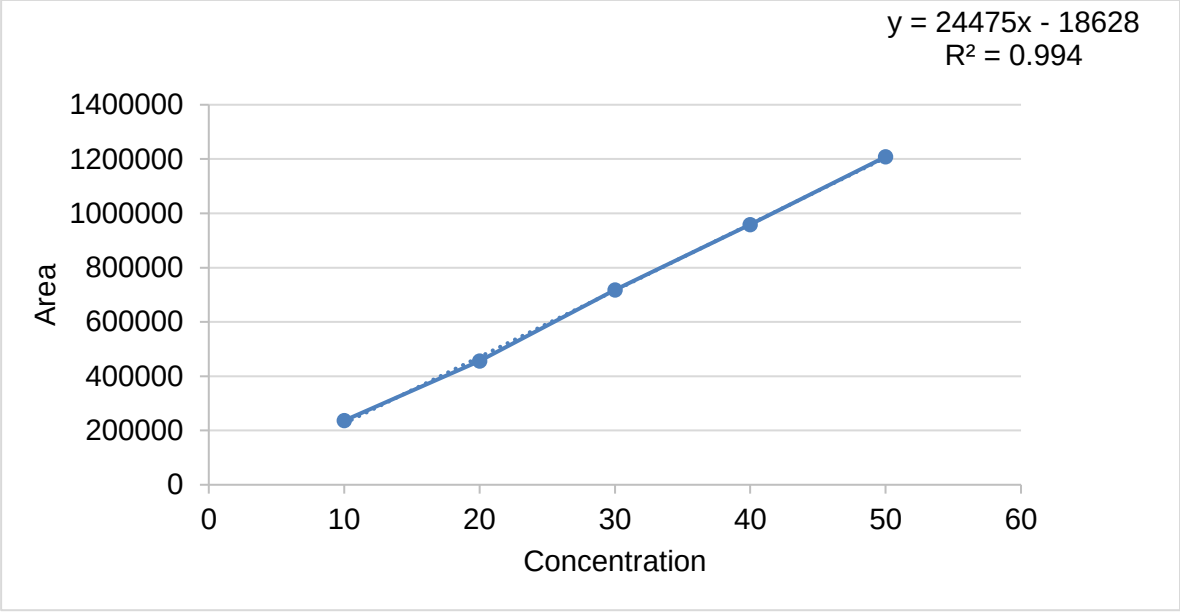

Fig 9. Calibration curve for Remogliflozin

Precision

To assess the repeatability, six samples of 100 µg/ml Remogliflozin etabonate was measured. The daily and the three interday precision measurements were done. The Remogliflozin concentrations used were 100, 150 and 200 µg/ml and the tests were done three times, on three different days and at 2 hour intervals on the same day. 2 was the least acceptable value for % RSD.

Interday		Area	Standard Deviation		Accuracy	Precision
Sr. No.	Conc.		Mean	SD	%SD	%RSD
1	30 30	718550 718631	715889	4679.310526	0.65363632	0.121971228

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	30	710486				
2	30 30 30	719418 718212 712933	716854.3333	3449.094132	0.48114296	

Table 11. Interday study of Remogliflozin

Intraday		Area	Standard Deviation		Accuracy	Precision
Sr. No.	Conc. (µg/mL)		Mean	SD	%SD	%RSD
1	30 30 30	718550 718631 710486	715889	4679.31053	0.6536363	0.26548513
2	30 30 30	719709 721605 717603	719639	2001.91808	0.2781837	

Table 12. Intraday study of Remogliflozin

Accuracy

According to the definitions accuracy is defined as the extent to which the measured value is close to the actual value. The recovery was done by performing the injection of 50%, 100% & 150% levels three times and then the Amount found, Amount added, %Recovery, Mean recovery and %RSD were calculated.

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery	Conc. Taken (µg/mL)	Conc. Found (µg/mL)
1	50% Recovery	718550	715031	99.51026373	30	29.85307912
2	100% Recovery	959210	957487	99.82037302	40	39.92814921
3	150% Recovery	1208350	1207729	99.94860761	50	49.9743038

Table 13. Accuracy data of Remogliflozin

LOD and LOQ- Following the formula, we were able to determine the LOD. Below, we will go over the outcome.

The limit of detection is equal to 3.3 times the standard deviation divided by the slope.

Limit of detection (LOD)= 0.32 µ/ml.

The following formula was used to determine the LOQ**.** Below, we will go over the outcome.

The limit of quantification (LOQ) is equal to10 standard errors of the estimate of the slope.

Limit of quantification = 0.09 µ/ml

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Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Remogliflozin	244.393	24475	0.032951865	0.0998541

Table 14. LOD and LOQ of Remogliflozin

Robustness studies

Robustness is defined as variations in the conditions of the parameters that should not in any way alter the performance of any method. Thus, the robustness was made by small alterations in the parameters of the change of ph and wavelength.

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%SD
1	20 20 20	455730 454249 458193	456057.33	1992.271	0.4368466

Table 15. Change in ph of Remogliflozin

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%SD
1	20 20 20	455730 460967 458534	458410.33	2620.689	0.5716907

Table 16. Change in wavelength of Remogliflozin

Assay procedure

Remogliflozin etabonate is a drug that can be easily bought from local pharmacies with the brand REMO®-ZEN with a strength of 100mg provided by Glenmark Pharma limited. One thousand micrograms per milliliter of the sample solution was prepared and then diluted to a concentration of 50 micrograms per milliliter and the later was injected into the HPLC system, the peak areas of the obtained chromatogram were noted down, and the percentage of assay calculated as shown in Table 17.

Sr. No.	Conc. (µg/mL)	Area of Standard	Area of Sample	% Assay
1	30	718550	718020	99.9262403

Table 17. Assay of Remogliflozin

Result

An efficient experimental design based on the Box-Behnken design of three key components of the RP-HPLC method (composition, flow rate, and wavelength) is presented. The chromatographic conditions were optimized with the Design Expert software 13.0.5.0 version, Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron), mobile phase used Methanol:water (90:10, v/v), and the flow rate was 0.9 ml/min with retention time 4.48 min. The developed method was linear with $R^2 = 0.9994$ for 10–50 µg/ml range at 225 nm detection wavelength.

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The system suitability test parameters- asymmetry factor, and theoretical plates were 1.30 and 8129. The % RSD for inter-day and intraday precision was found to be 0.1219 and 0.2654 respectively. The robustness values were less than 2%. The assay was found to be 99.92%. The method validation parameters were within the prescribed limit as per ICH guidelines

Conclusion

Ultimately, the research proved that the Box-Behnken design was the most effective experimental framework for optimizing the RP-HPLC method's composition, flow rate, and wavelength. At a flow rate of 0.9 ml/min and a mobile phase composition of 90:10, v/v, the chromatographic system parameters were optimized using Design Expert software version 13.0.5.0. The column employed was a Cosmosil C18 with dimensions of 250mm x 4.6ID. The material had a primary particle size of 5 microns and a retention time of 4.48 min. The introduced approach exhibited a strong linear correlation with a coefficient of determination of 0.9994 at a wavelength of 225 nm when recognized, within the optimal concentration range of 10-50 µg/ml. The asymmetry factor of this structure is 1.29 and theoretical plates amounting to 7344, the test conditions for system suitability gave favourable results. Both the inter and intraday precision measures, as measured by the percentage of RSD, had very low variability, coming in at 0.1219% and 0.2654%, respectively. Stability with variation within 2% was revealed via robustness testing. Furthermore, a high percentage of 99.92% was produced by the assay, which suggests that the quantification was accurate. The RP-HPLC technique was confirmed to be reliable and robust for the intended analysis because the validation parameters met the prescribed limits according to ICH recommendations.

Abbreviations

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography.

LOD: Limit of Detection.

LOQ: Limit of Quantification.

R.T: Retention time

µg/ml: Microgram per milli-liter

ICH: International Conference on Harmonization

Acknowledgements

Ethics approval and consent to participate: we ensure the originality and integrity of this article.

Consent for publication: We confirm that all individuals featured in this research article have provided explicit consent for using their data, images, or identities.

Availability of data and material: We pledge to make the data and materials underlying this article available upon request, in compliance with the journal's policies and ethical considerations.

Competing Interests: The authors declare no competing interests that could influence the interpretation or presentation of the research article's findings.

Funding Source: No

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