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Rp-Hplc Method Development and Validation of Sotagliflozin

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ABSTRACT: The present study aimed to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of sotagliflozin. A Quality by Design (QbD)-based approach using response surface methodology and a three-factor, three-level Box–Behnken design was employed to optimize the chromatographic conditions. Chromatographic separation was performed using a C18 column with a mobile phase consisting of methanol and water in a ratio of 80:20 (v/v). The flow rate was maintained at 1.0 mL/min, with UV detection at 232 nm and an injection volume of 20 µL. The total run time was 10 minutes. The developed method was evaluated for linearity, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Linearity was established over a concentration range of 2–12 ppm, with a coefficient of determination (R^2) of 0.997. System precision and method precision demonstrated %RSD values of 0.406% and 0.147%, respectively. Intraday and interday precision studies showed %RSD values below 1%, indicating good reproducibility. Robustness studies demonstrated acceptable performance following minor variations in wavelength and injection volume. The LOD and LOQ were determined to be 0.61 ppm and 1.86 ppm, respectively. Overall, the developed RP-HPLC method demonstrated satisfactory linearity, precision, sensitivity, and robustness, indicating its potential suitability for the routine quantitative analysis of sotagliflozin in pharmaceutical quality-control laboratories.

1. INTRODUCTION

Sotagliflozin is an orally administered antidiabetic agent belonging to the class of sodium-glucose cotransporter inhibitors. It inhibits both sodium-glucose cotransporter 1 (SGLT1) and sodium-glucose cotransporter 2 (SGLT2), which are involved in glucose absorption and reabsorption, respectively. [1-2] SGLT1 is primarily responsible for glucose and galactose absorption in the intestine, whereas SGLT2 plays a major role in glucose reabsorption in the renal proximal tubules. By inhibiting these transporters, sotagliflozin reduces intestinal glucose absorption and increases urinary glucose excretion, thereby contributing to the regulation of blood glucose levels. It is used as an adjunct to diet and exercise in adults with type 2 diabetes mellitus and has also been approved for specific cardiovascular and renal indications in selected patient populations. [3-5]

The growing importance of sotagliflozin in the management of metabolic and cardiorenal disorders highlights the need for reliable analytical methods for its estimation in pharmaceutical formulations.[6] Analytical method development plays a significant role in pharmaceutical research by establishing suitable conditions for the accurate, precise, and reproducible determination of active pharmaceutical ingredients. Among the various analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) is widely used for pharmaceutical analysis because of its high separation efficiency, reproducibility, sensitivity, and compatibility with routine quality-control procedures.[7-10]

RP-HPLC separates compounds based on their differential distribution between a non-polar stationary phase and a relatively polar mobile phase. The chromatographic performance depends on several parameters, including the stationary phase, mobile-phase composition, pH, flow rate, detection wavelength, injection volume, and column temperature. Appropriate optimization of these parameters is essential to obtain satisfactory peak shape, suitable retention time, adequate theoretical plate count, and acceptable chromatographic resolution. For the analysis of sotagliflozin, developing an optimized RP-HPLC method can facilitate its reliable identification and quantitative determination in bulk drug and pharmaceutical dosage forms.[11-13]

Method validation is an essential component of analytical procedure development and is performed to demonstrate that the proposed method is suitable for its intended purpose. Validation parameters are generally evaluated in accordance with the International Council for Harmonisation (ICH) Q2 (R2) guideline and may include specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, and robustness, as applicable to the analytical procedure. System suitability testing is also performed to verify the satisfactory performance of the chromatographic system before sample analysis. Evaluation of these parameters establishes the reliability and reproducibility of the developed method for routine pharmaceutical analysis. [14-15]

The present study aims to develop and validate a simple, accurate, precise, and reproducible RP-HPLC method for the quantitative estimation of Sotagliflozin. The chromatographic conditions are optimized to achieve suitable separation and consistent analytical performance. The developed method is evaluated for the relevant validation parameters to establish its suitability for the quantitative determination of Sotagliflozin in bulk drug and, where applicable, pharmaceutical dosage forms. The proposed analytical procedure is intended to provide a reliable approach for routine quality-control analysis and further analytical investigations of Sotagliflozin.

2. MATERIAL AND METHODS

2.1 MATERIAL

Sotagliflozin API was procured from B L Chemicals, Mumbai, India. HPLC-grade methanol, acetonitrile, and water were procured from Advent, India. All chemicals and solvents used in the study were of analytical or HPLC grade, as applicable.

2.2 Instruments

The validation inquiry necessitated essential equipment and supplies, including a mobile phase filtration apparatus and an analytical balance. The HPLC system utilized Jasco ChromNAV 2.0 chromatography software, a Jasco 4600 Separation Module, and a Jasco UV detector. Validation studies were conducted utilizing both standard and test samples.

2.3 Chromatographic condition

The chromatographic separation of sotagliflozin was performed using a Finepack Jasco C18 column (5 µm, 4.6 × 250 mm) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30 ± 5°C, while the sample temperature was maintained at 25 ± 5°C. The injection volume was 20 µL, and detection was carried out using a UV detector at 232 nm. The total chromatographic run time was 10 minutes, with sotagliflozin exhibiting an approximate retention time of 3.6 minutes under the specified chromatographic conditions.

2.4 Optimization

The experimental design was developed using response surface methodology (RSM) under the Quality by Design (QbD) approach to optimize the RP-HPLC method for sotagliflozin with a minimum number of experimental runs. A three-factor, three-level Box–Behnken design (BBD) was employed to evaluate the effects of methanol composition (A), water composition (B), and flow rate (C) on the chromatographic responses. The independent variables were studied at three levels: low (−1), medium (0), and high (+1). The dependent variables selected for optimization were retention time (Y_1), symmetric factor (Y_2), and theoretical plate count (Y_3). Retention time was targeted for minimization, symmetric factor was targeted toward an acceptable value close to 1, and theoretical plate count was targeted for maximization. The design enabled the assessment of the individual, interaction, and quadratic effects of the selected factors on the chromatographic performance of sotagliflozin. [17]

2.5 Method validation

2.5.1 Linearity

A standard stock solution of sotagliflozin was prepared by accurately weighing 10 mg of the drug and dissolving it in methanol in a 10 mL volumetric flask to obtain a concentration of 1000 ppm. From this stock solution, 1 mL was transferred into a 10 mL volumetric flask and diluted to the mark with the selected diluent to obtain a 100 ppm intermediate stock solution. The intermediate stock solution was further diluted appropriately to prepare six working standard solutions at concentrations of 2, 4, 6, 8, 10, and 12 ppm. Each solution was analyzed using the optimized RP-HPLC conditions, and the corresponding peak areas were recorded for the linearity study.[18]

2.5.2 Precision

2.5.2.1 System precision

System precision was evaluated from six replicate injections of standard as per proposed method.

2.5.2.2 Method Precision

The six test solutions were prepared separately. Each was analyzed as per proposed procedure.

2.5.2.3 Intermediate precision

The six test solutions were prepared separately. Each was analyzed as per proposed procedure (analyzed in morning, afternoon and evening i.e. intraday precision and today, tomorrow and day after tomorrow i.e. interday precision). [19-20]

2.5.3 Robustness

The influences of slightly changed parameters of the chromatographic conditions were tested according to ICH guidelines to demonstrate robustness of the method. The tests are carried out by injecting Blank and standard solution by varying same of the parameters of chromatography mentioned below.[21-22]

Table 1: Robustness parameter of Sotagliflozin

Sr. No	Parameters	Working parameter	- changes	+ changes
1	Wavelength	232 nm	230 nm	234 nm
2	Injection volume	20 µL	18 µL	22 µL

2.5.4 Limit of Detection and Quantification

Limit of detection and quantification explains the sensitivity of the analytical techniques. Hence, LOD and LOQ were determined for both the methods using 3.3 d/s and 10 d/s equations, respectively, where "d" is the standard deviation of the response intercept and "s" is the slope of the standardization curve.[23-24]

3. RESULT

3.1 Optimization of Chromatographic Conditions

Table 2: Optimization of Chromatographic Parameters Using Design-Expert with Desirability Approach

Run	Mobile phase Composition (Methanol) (%)	Mobile phase composition (Water) (%)	Flow Rate(mL/min)	Retention Time (min)	Symmetry Factor	Theoretical plate
1	80	17.5	1	3.167	1.22	3950
2	80	20	0.9	3.1257	1.234	3985

3	77.5	17.5	0.9	3.087	1.199	4010
4	75	17.5	0.8	3.0522	1.243	3880
5	75	17.5	1	3.092	1.185	4025
6	77.5	20	1	3.142	1.186	4045
7	80	20	1	3.167	1.181	4079
8	80	17.5	0.8	3.063	1.236	3920
9	80	15	0.9	3.024	1.268	3855
10	75	15	0.9	3.025	1.281	3805
11	75	20	0.9	3.115	1.213	3965
12	77.5	15	1	3.043	1.256	3875
13	77.5	15	0.8	3.013	1.277	3830

3.1.1 Retention Time

The ANOVA results for the linear model of Response 1 (Retention Time) indicate that the model was statistically significant, with an F-value of 26.28 and a p-value of less than 0.0001. Among the independent variables, mobile phase composition (water, B) and flow rate (C) were significant factors, with p-values of 0.0001 and 0.0059, respectively, whereas methanol composition (A) was not significant (p = 0.1111). The coefficient of determination (R^2) was 0.8975, indicating that the model explained 89.75% of the variability in retention time. The adjusted R^2 and predicted R^2 values were 0.8634 and 0.7987, respectively, showing reasonable agreement between the model fit and predictive ability. The coefficient of variation (CV) was 0.6481%, indicating low variability relative to the mean response. Furthermore, the adequate precision value of 14.4545 was greater than 4, indicating an adequate signal for navigating the design space. Overall, the model demonstrated satisfactory statistical significance and can be used to evaluate the effects of the selected chromatographic factors on sotagliflozin retention time within the studied experimental range.

Final Equation in Terms of Coded Factors

Retention Time = 3.08 + 0.0119A + 0.0474B + 0.0268C

Final Equation in Terms of Actual Factors

Retention Time = 2.13747 + 0.004778 Mobile Phase Composition (Methanol) + 0.018957(Mobile Phase Composition (Water) + 0.268102(Flow Rate)

3.1.2 Symmetry Factor

The ANOVA results for the linear model of Response 2 (Symmetry Factor) indicate that the model was statistically significant, with an F-value of 8.77 and a p-value of 0.0049, suggesting that the model adequately describes the relationship between the selected factors and the response. Among the independent variables, mobile phase composition (water, B) was a significant model term (p = 0.0034), whereas methanol composition (A) and flow rate (C) were not significant, with p-values of 0.6190 and 0.0552, respectively. The results indicate that water composition had a statistically significant effect on the symmetry factor within the investigated experimental range. Although the overall model was significant, the effects of the individual factors should be interpreted according to their respective p-values.

Final Equation in Terms of Coded Factors

Symmetry Factor = 1.23 + 0.0036A - 0.0297B - 0.0169C

Final Equation in Terms of Actual Factors

Symmetry Factor =+1.48083+0.001428 Mobile Phase Composition (Methanol)-0.011890(Mobile Phase Composition (Water) -0.168842(Flow Rate)

3.1.3 Theoretical plate

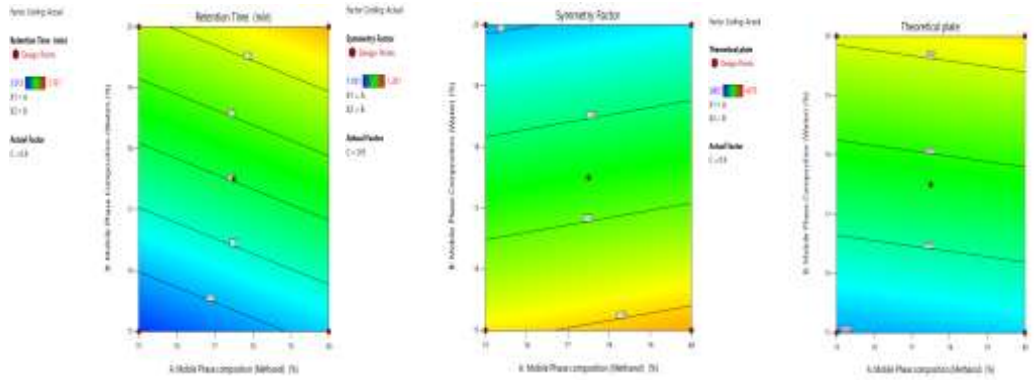
The ANOVA results for the theoretical plate response indicate that the linear model is significant, with an F-value of 13.70 and a p-value of 0.0011. Among the independent variables, mobile phase composition (water) (B) and flow rate (C) significantly affected the theoretical plate response, with p-values of 0.0008 and 0.0362, respectively. Methanol composition (A) was not significant (p = 0.6381). The model R^2 value of 0.8204 indicates that 82.04% of the variability in theoretical plate values was explained by the model. The adjusted R^2 was 0.7605, while the predicted R^2 was 0.6677. The coefficient of variation (CV) of 1.08% indicates relatively low variability, and the adequate precision value of 10.2079, which exceeds 4, indicates an adequate signal-to-noise ratio for navigating the design space.

Final equation in terms of coded factors

Theoretical Plate =+3933.74+7.01A +77.95B+39.20C

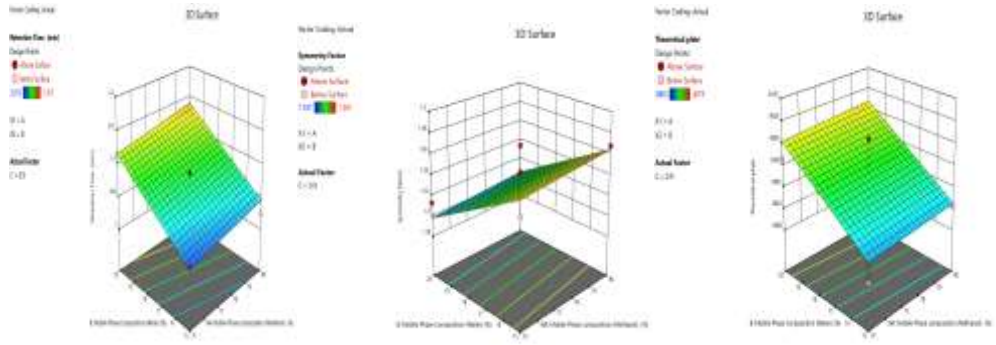
Final equation in terms of actual factors

Theoretical Plate =+2817.89395+2.80501(Methanol) +31.17916(Water) +392.02170(Flow Rate)



(A) **(B)** **(C)**

Figure 1: A- Contour plots showing the effects of independent variables on retention time, B-Contour plots showing the effects of independent variables on Symmetry factor, C-Contour plots showing the effects of independent variables on theoretical plate.



(A) **(B)** **(C)**

Figure 2: A- 3D Surface showing the effects of independent variables on retention time, B-3D Surface showing the effects of independent variables on Symmetry factor, C-3D Surface showing the effects of independent variables on theoretical plate.

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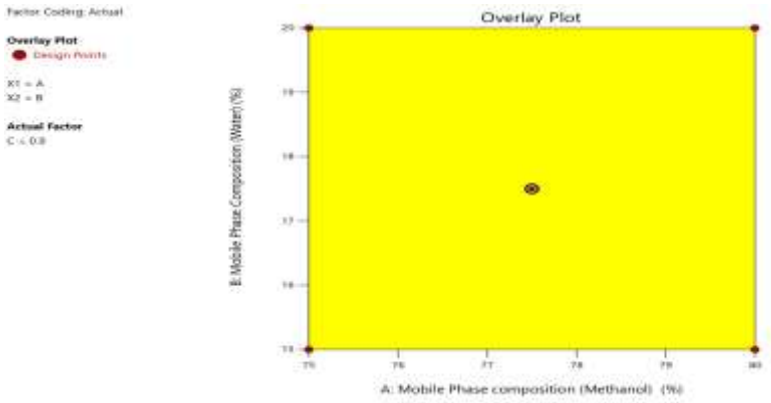


Figure 3: Overlay plot AQbD (method operable design region).

3.2 Linearity

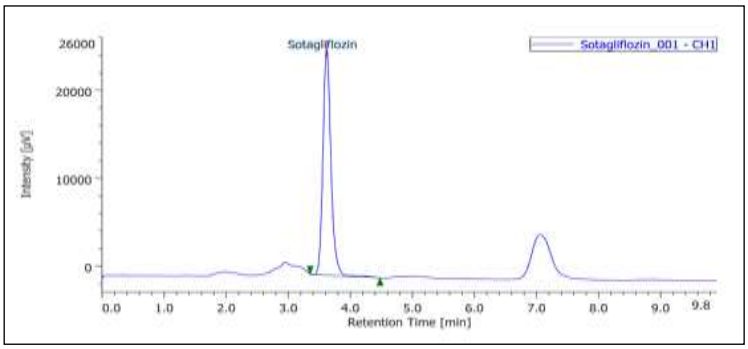


Figure 4: HPLC chromatogram of Sotagliflozin standard solution (2 ppm) for linearity study.

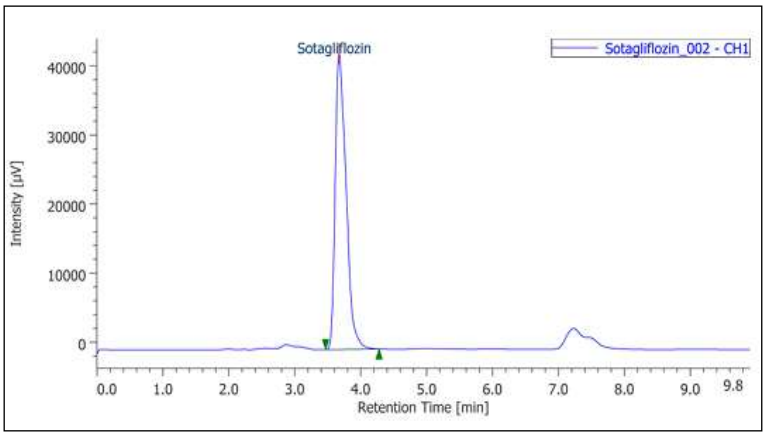


Figure 5: HPLC chromatogram of Sotagliflozin standard solution (4 ppm) for linearity study.

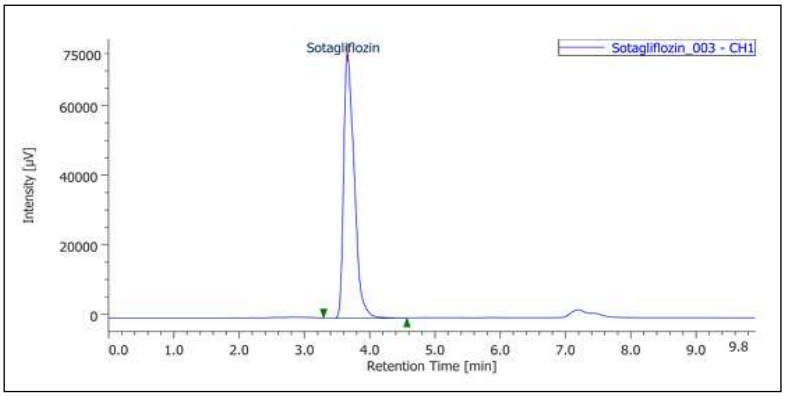


Figure 6: HPLC chromatogram of Sotagliflozin standard solution (6 ppm) for linearity study.

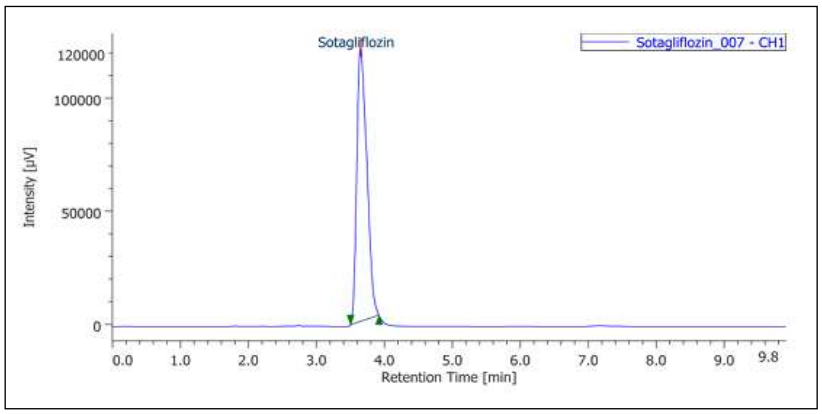


Figure 7: HPLC chromatogram of Sotagliflozin standard solution (8 ppm) for linearity study.

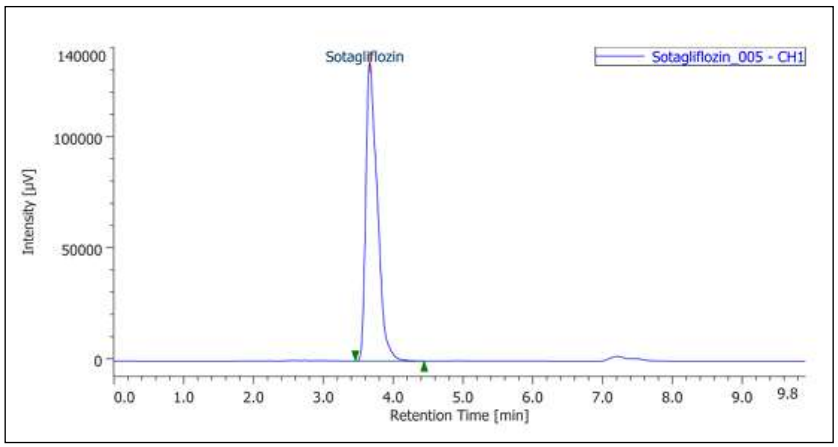


Figure 8: HPLC chromatogram of Sotagliflozin standard solution (10 ppm) for linearity study.

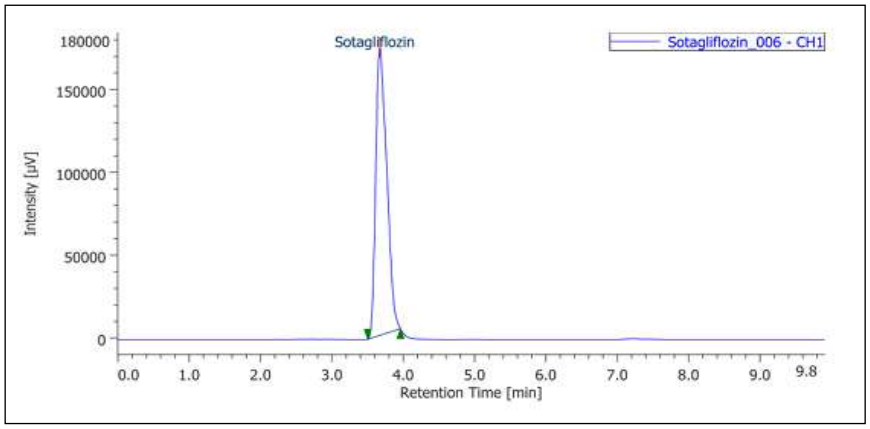


Figure 9 :HPLC chromatogram of Sotagliflozin standard solution (12 ppm) for linearity study.

Table 3: Linearity of Sotagliflozin

Conc. of Sample (ppm)	**Average Peak area**
02	230415
04	534166
06	856493
08	1239832
10	1541996
12	1861336
0.997	

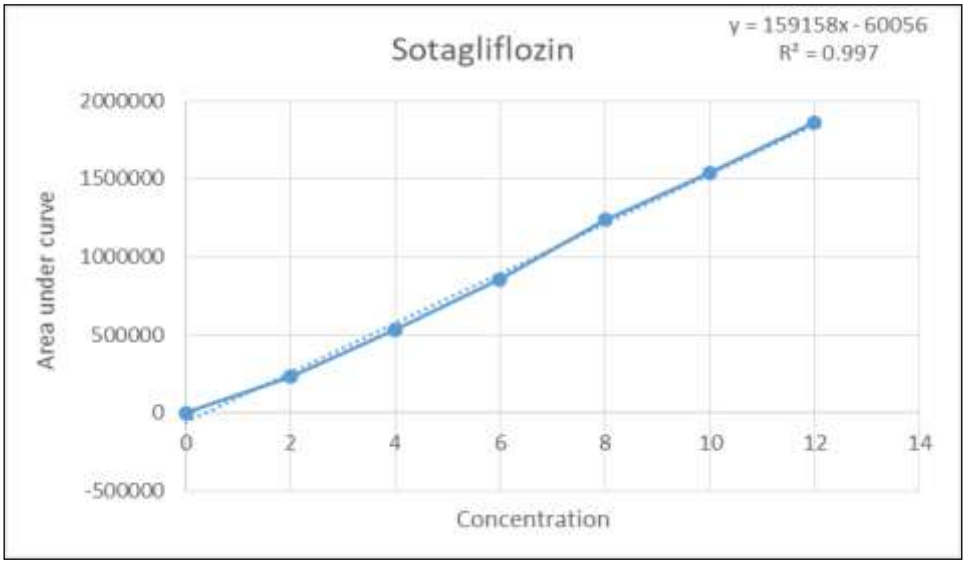


Figure 10: Linearity Graph of Sotagliflozin

The linearity study of sotagliflozin was performed over the concentration range of 2–12 ppm. The average peak area increased from 230415 at 2 ppm to 1861336 at 12 ppm, demonstrating an increase in detector response with increasing concentration. The coefficient of determination ((R2 = 0.997) indicates a strong linear relationship between sotagliflozin concentration and peak area over the studied range. These results support the linearity of the developed analytical method within the evaluated concentration range.

3.3 Precision

3.3.1 System precision

Table 4: System Precision of Sotagliflozin

Inject. No	Peak Area of Sotaglifliozin
1	1295329
2	1280359
3	1285489
4	1288167
5	1283611
6	1283423
Mean	1286063
% RSD	0.406%

The system precision study of sotagliflozin was performed by six replicate injections of the standard solution. The mean peak area was 1,286,063, with a %RSD of 0.406%. Since the %RSD was below 2%, the results indicate good injection repeatability and acceptable system precision under the specified chromatographic conditions.

3.3.2 Method precision

Table 5: Method Precision of Sotagliflozin

Sample No.	% Assay of Sample
1	98.64
2	98.22
3	98.51
4	98.54
5	98.57
6	98.53
Mean	98.501
% RSD	0.147%

The method precision study of sotagliflozin was evaluated using six replicate sample measurements. The mean assay value was 98.502%, with a %RSD of approximately 0.147%. The %RSD was below 2%, indicating good repeatability and acceptable precision of the developed analytical method under the specified experimental conditions.

3.3.3 Intermediate precision

Table 6: Intraday Precision of Sotagliflozin at Different Time Intervals

Name of Analyte	Time interval	Intraday	Average	% RSD
Sotagliflozin	Morning	783922	783833	0.370%
	Morning	786690		
	Morning	780888		
	Afternoon	770872	771451	0.657%
	Afternoon	776786		
	Afternoon	766695		
	Evening	779246	780220	0.468%
	Evening	777153		
	Evening	784262		

Table 7 :Interday Precision of Sotagliflozin at Different Time Intervals

Name of Analyte	Time interval	Interday	Average	% RSD
Sotagliflozin	Today	1274675	1274887	0.214%
	Today	1277718		
	Today	1272268		
	Tomorrow	1273077	1283729	0.785%
	Tomorrow	1285010		
	Tomorrow	1293102		
	Day after tomorrow	1301805	1304933	0.251%
	Day after tomorrow	1308328		
	Day after tomorrow	1304668		

The intermediate precision study of sotagliflozin demonstrated good reproducibility under intraday and interday conditions. The intraday %RSD values ranged from 0.370% to 0.657%, while the interday %RSD values ranged from 0.214% to 0.785%. All %RSD values were below 2%, indicating acceptable precision and repeatability of the analytical method under the evaluated conditions.

3.1 Robustness

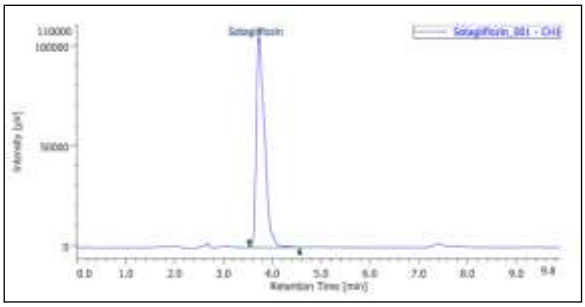


Figure 11: Robustness (Wavelength -2 nm) 1

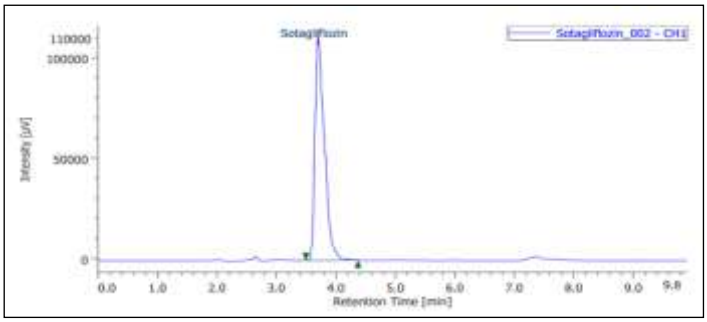


Figure 12: Robustness (Wavelength -2 nm) 2

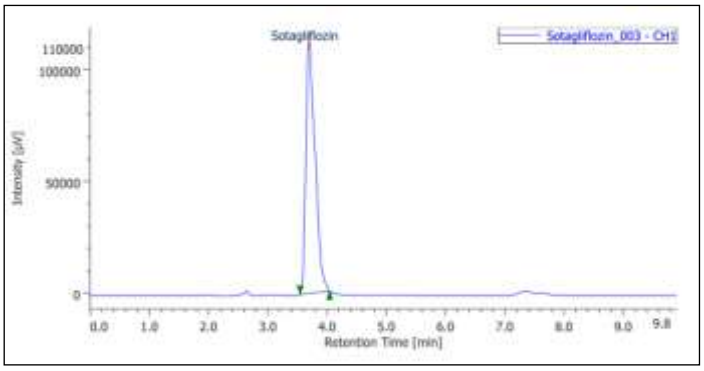


Figure 13: Robustness (Wavelength -2 nm) 3

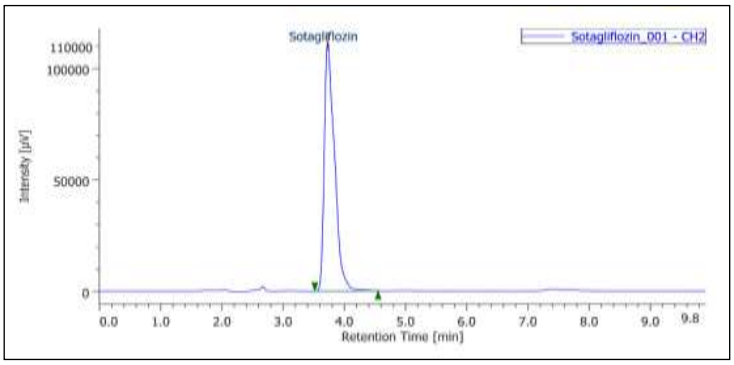


Figure 14: Robustness (Wavelength +2 nm) 1

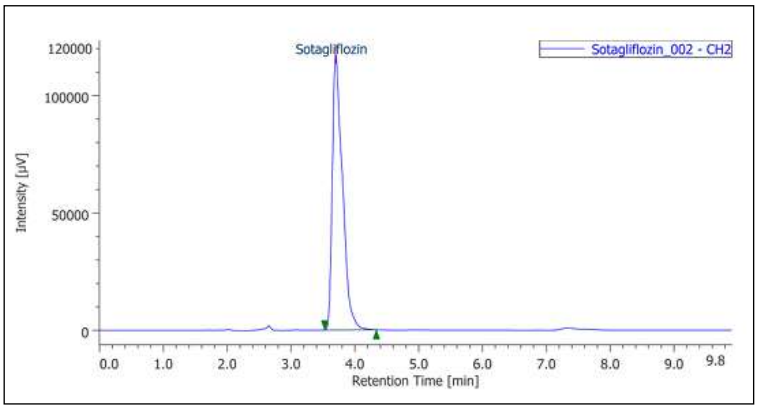


Figure 15: Robustness (Wavelength +2 nm) 2

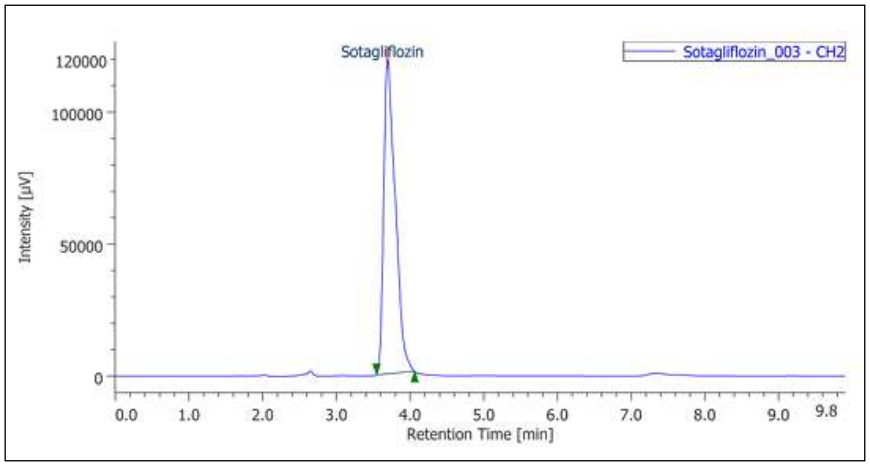


Figure 16: Robustness (Wavelength +2 nm) 3

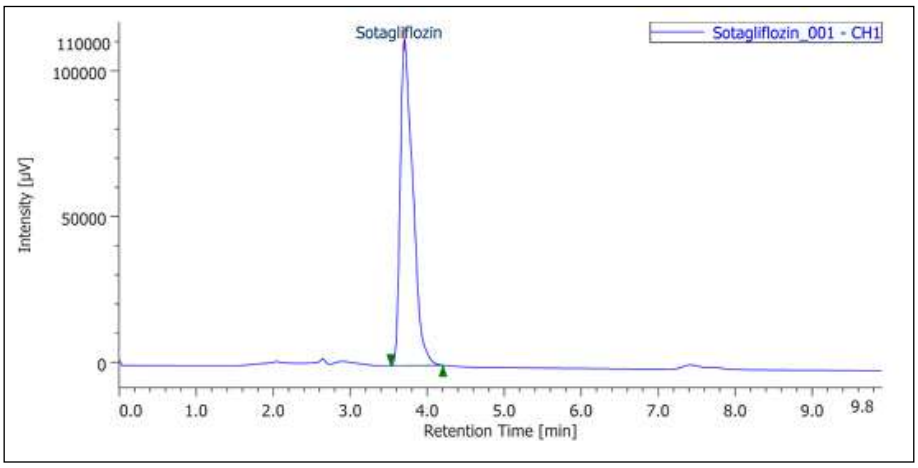


Figure 17: Robustness (Injection volume -2 µL) 1

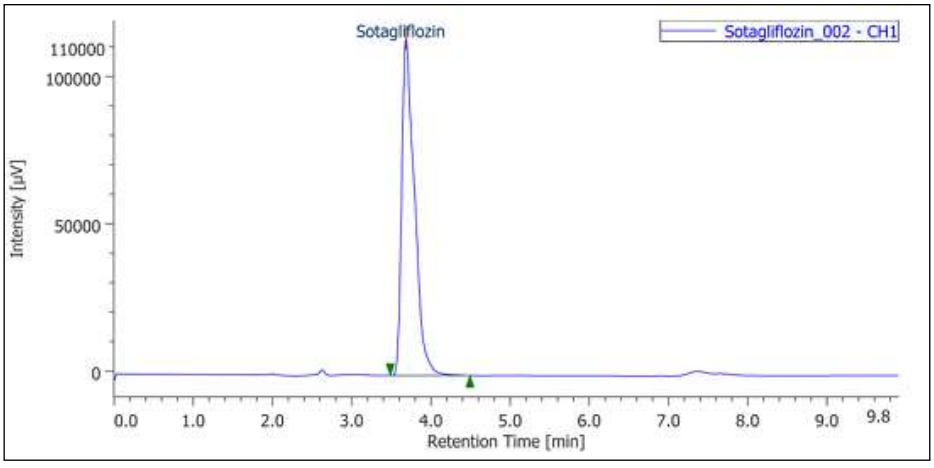


Figure 18: Robustness (Injection volume -2 µL) 2

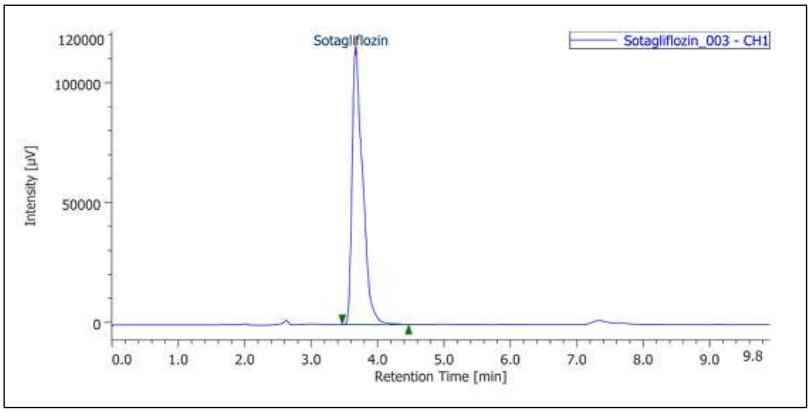


Figure 19: Robustness (Injection volume -2 µL) 3

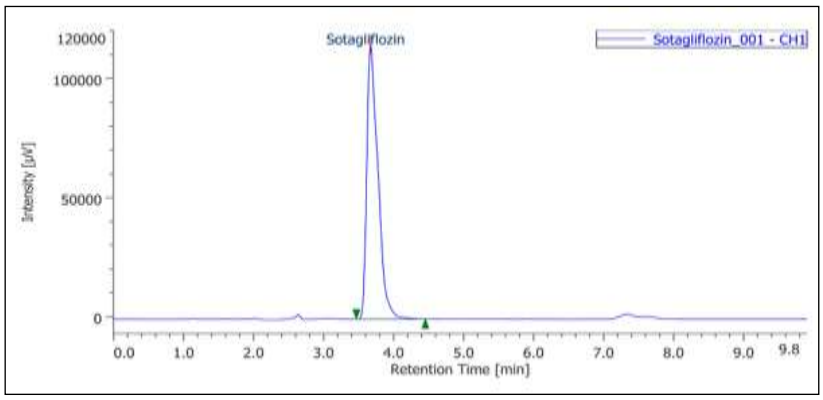


Figure 20 : Robustness (Injection volume +2 µL) 1

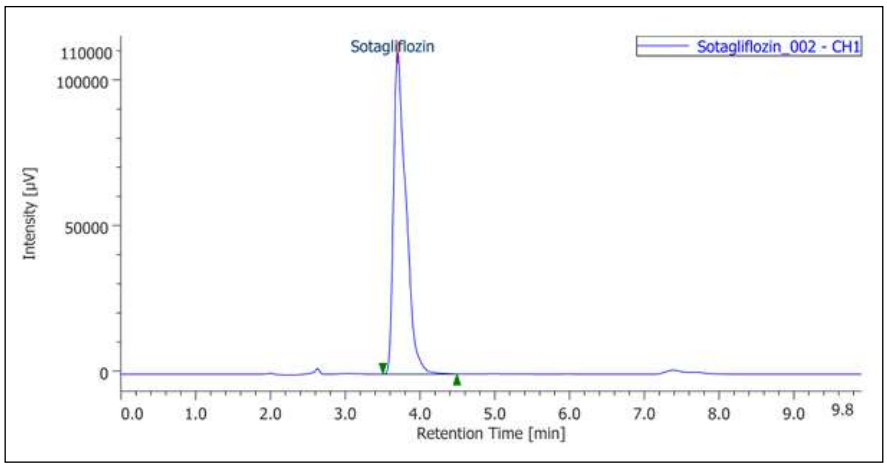


Figure 21: Robustness (Injection volume +2 µL) 2

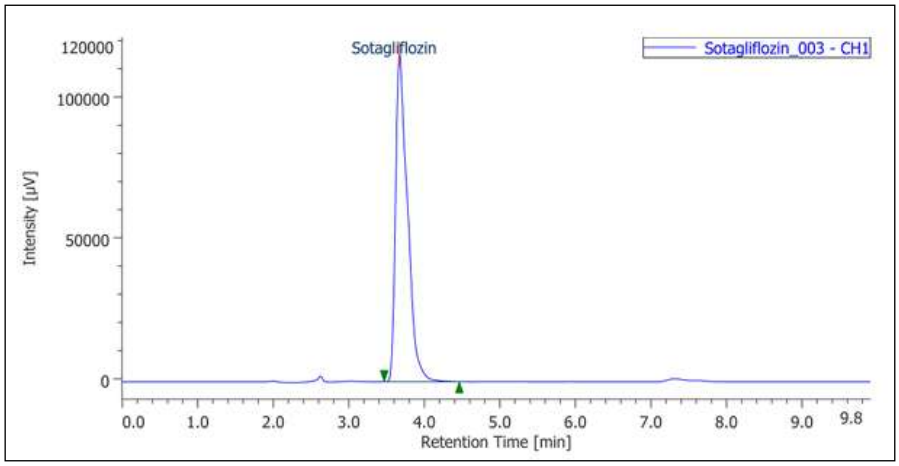


Figure 22: Robustness (Injection volume +2 µL) 3

Table 8: Robustness of Sotagliflozin

Robustness parameters		% RSD	Peak tailing	Theoretical plates	Remark
Sample					
Wavelength (-2 nm) and (+2 nm)	1227993	0.360%	1.723	2272	Pass
	1227835		1.660	2479	Pass
	1220282		1.640	2511	Pass
	1296706	0.156%	1.704	2265	Pass
	1294277		1.670	2481	Pass
	1298301		1.648	2425	Pass
Injection volume (-2 µL) and (+2 µL)	1271431	0.301%	1.699	2279	Pass
	1277685		1.697	2440	Pass
	1278432		1.723	2505	Pass
	1276948	0.017%	1.706	2383	Pass
	1276920		1.679	2221	Pass
	1277319		1.759	2482	Pass

3.2 LOD & LOQ

Table 9: LOD & LOQ Parameters of Sotagliflozin

Parameter	Value	Acceptance Criteria	Remark
LOD	0.61 ppm	Signal-to-Noise (S/N) ≥ **3:1** or calculated using **3.3σ/S**	Acceptable
LOQ	1.86 ppm	Signal-to-Noise (S/N) ≥ **10:1** or calculated using **10σ/S**	Acceptable

The limit of detection (LOD) and limit of quantification (LOQ) of sotagliflozin were found to be 0.61 ppm and 1.86 ppm, respectively. The values indicate that the developed analytical method can detect sotagliflozin at low concentrations and quantify it at concentrations above the detection limit. The reported values are consistent with the relationship between LOD and LOQ, with LOQ higher than LOD. The acceptance criteria should be confirmed using the experimental signal-to-noise ratios or calculations based on the standard deviation of the response and the slope of the calibration curve.

CONCLUSION

The present study successfully developed and evaluated a Quality by Design-based RP-HPLC method for the quantitative estimation of sotagliflozin. The Box–Behnken experimental design facilitated the systematic optimization of chromatographic parameters, including mobile-phase composition and flow rate. The developed method demonstrated good linearity over the concentration range of 2–12 ppm, with an R^2 value of 0.997. System precision and method precision were satisfactory, with %RSD values of 0.406% and 0.147%, respectively. Intraday and interday precision results further indicated good reproducibility. Robustness studies showed acceptable chromatographic performance following minor changes in wavelength and injection volume. The LOD and LOQ were found to be 0.61 ppm and 1.86 ppm, respectively, demonstrating the method's sensitivity. Therefore, the developed method offers a simple, precise, reproducible, and sensitive analytical approach for the quantitative determination of sotagliflozin. Further evaluation of accuracy, specificity, and additional validation parameters would strengthen its suitability for routine pharmaceutical quality-control applications.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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