Brazilian Journal of Analytical Chemistry
Pre-publication – Accepted after peer review
doi: 10.30744/brjac.2179-3425.AR-22-2026

ARTICLE

Implementation of Analytical Quality by Design to Develop a Stability-Indicating RP-HPLC Method for Sotagliflozin

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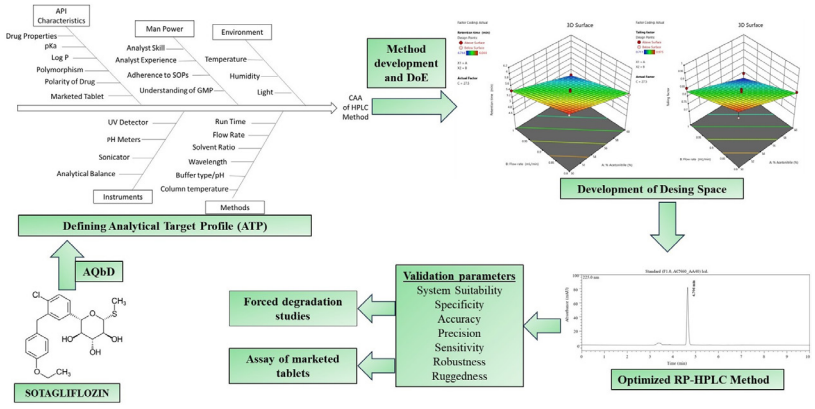

This study applied Analytical Quality by Design (AQbD) approaches to systematically develop a stability-indicating RP-HPLC method for the quantification of sotagliflozin in both bulk drug substance and tablet formulations. The Analytical Target Profile (ATP) was determined to guide the selection of Critical Analytical Attributes (CAAs), namely retention time and tailing factor. The Critical Method Parameters (CMPs) including mobile phase composition, flow rate, and column temperature were optimized using a Box-Behnken design to effectively control the CAAs. Chromatographic separation employed a mobile phase of acetonitrile and 10 mM ammonium acetate buffer in a 60:40 (v/v) ratio. An isocratic mobile phase, adjusted to pH 3.0 with 0.05% ortho-phosphoric acid, was delivered at 1.0 mL min^{-1} through an ODS C18 column (250 mm × 4.6 mm i.d., 5 µm) maintained at 28 °C. Detection was conducted at 225 nm, corresponding to the λ_{max} of sotagliflozin. Analysis of variance (ANOVA) test resulted in statistically significant linear model terms ($p < 0.05$). The developed method was validated following ICH guidelines which demonstrated linearity over 5-25 µg mL^{-1} ($R^2 = 0.999$), with LOD and LOQ of 0.12 and 0.38 µg mL^{-1}, respectively. Forced degradation studies supported the stability-indicating nature of the method, with marked degradation under acidic (26.45%), alkaline (29.29%), and thermal (23.58%) conditions, while greater stability was observed under oxidative, hydrolytic, and photolytic stress. Therefore, this AQbD-based stability-indicating RP-HPLC method provides a robust and scientifically justified approach for the routine analysis of sotagliflozin, supporting reliable drug product stability assessment.

Keywords: AQbD, stability-indicating, RP-HPLC, sotagliflozin, ICH, CAAs

Cite: Hossain, Md. J.; Islam, M. R.; Chowdhury, J. A.; Akhter, L.; Haque, Md. A.; Akter, S.; Islam, Md. Z. Implementation of Analytical Quality by Design to Develop a Stability-Indicating RP-HPLC Method for Sotagliflozin. *Braz. J. Anal. Chem.* (Forthcoming). https://doi.org/10.30744/brjac.2179-3425.AR-22-2026

Submitted: March 4, 2026; **Revised:** April 27, 2026; June 1, 2026; **Accepted:** June 6, 2026; **Published online:** July 2026.

INTRODUCTION

Diabetes is a critical public health concern, with the International Diabetes Federation forecasting an increase in affected individuals from 537 million in 2021 to 784 million by 2045.[1,2] Sotagliflozin is a novel antidiabetic drug that exhibits a dual inhibitory action on sodium-glucose cotransporters SGLT1 and SGLT2.[3] By decreasing intestinal glucose absorption and improving renal glucose excretion, it renders effective glycemic control in both fasting and postprandial states.[4,5] Approved by the USFDA in 2024, sotagliflozin also provides the advantages in lessening heart and kidney diseases.[6-8]

Several analytical methods, including UV spectrometry,[9,10] UPLC,[11,12] and LC-MS/MS,[13] have been developed for sotagliflozin quantification. These methods rely entirely on one-factor-at-a-time optimization without any DoE-based follow-up to study factor interactions or define a design space. For instance, Karanam *et al.* proposed a comparative UV and RP-HPLC method optimized via traditional one-factor-at-a-time (OFAT) trials, achieving good linearity and recovery.[10] Nonetheless, this empirical strategy provides little insight into method robustness and factor interactions.[14,15] In contrast, our present work used OFAT only for initial screening of solvent type, buffer concentration, pH, and stationary phase. The critical continuous variables (acetonitrile percentage, flow rate, and temperature) were then optimized using a Box-Behnken design. Sivakumar *et al.* showed a stability-indicating UPLC method offering faster analysis but without a specified risk-based strategy to demonstrate a robust design space.[11] The UPLC method by Chatterjee *et al*. lacks systematic optimization, resulting in shorter retention of sotagliflozin (t_R = 0.516 min) and raising concerns over selectivity.[12] Overall, these gaps underscore the need for a more structured development paradigm.

Analytical Quality by Design (AQbD) provides a structured framework that improves robustness, process understanding and supports structured risk management.[16] The AQbD workflow generally begins with defining the Analytical Target Profile (ATP), which defines the performance requirements of the method.[17-19] A generic representation of this stepwise development pathway is shown in Figure 1, depicting the transition from problem definition to method control and lifecycle management.

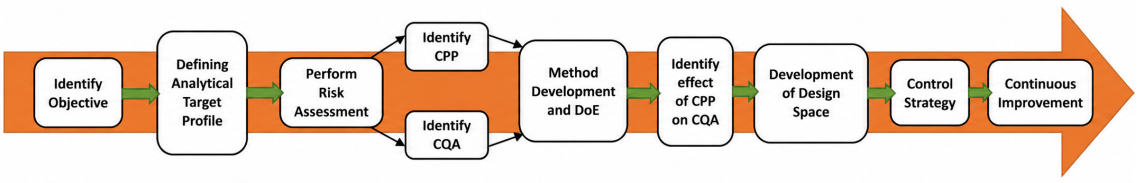

Figure 1. The typical sequence of steps in a QbD-based method development workflow.

Following ATP definition, Critical Method Parameters (CMPs) and their influences on Critical Analytical Attributes (CAAs) are identified using risk assessment tools such as Cause-and-Effect diagrams, Failure Mode and Effects Analysis (FMEA), and risk ranking approaches,[20-22] leading to systematic optimization to establish a Method Operable Design Region (MODR) for inherent robustness. In this research, the risk prioritization outcome is displayed in Figure 2, highlighting acetonitrile percentage, flow rate, and column temperature as the most predominant variables. These AQbD principles align closely with the regulatory expectations outlined in ICH Q8 (R2) and are further reinforced by the forthcoming ICH Q14 guideline, which formally incorporates AQbD principles into analytical method development.[23-25] Current literature reveals that reported chromatographic methods for sotagliflozin were established without utilizing a QbD-based methodology.[10-12] Therefore, this study employed AQbD principles to develop a specific, stability-indicating RP-HPLC method for sotagliflozin following ICH Q1A (R2).[26,27]

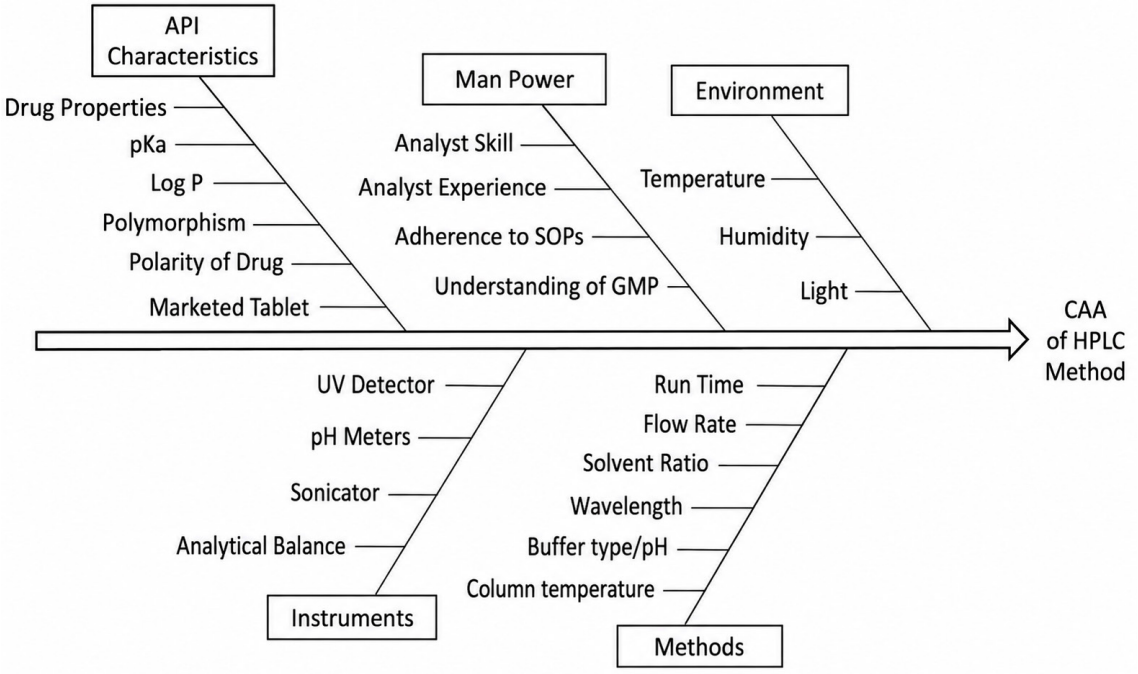

Figure 2. Cause-and-Effect analysis of Critical Method Parameters using a Fishbone diagram.

MATERIALS AND METHODS

Instrumentation

RP-HPLC analysis was performed using a Shimadzu LC-2050 series equipped along a gradient mixer and degasser, operated via LabSolutions software platform. An Elmasonic 2.8 L sonicator and a digital pH meter were employed for sample preparation. Sample injections were made using an injector with a 20.0 µL external loop and filtered all solutions through 0.45 µm Millipore™ membrane filters (Merck Ltd., Germany).

Reagent and materials

Sotagliflozin (purity 99.98%) was generously given as a gift by Incepta Pharmaceuticals Ltd., Bangladesh. The HPLC-grade acetonitrile (purity 99.80%) was procured from Sigma-Aldrich. Milli-Q distilled water of high purity was also sourced from Sigma-Aldrich. For analysis, Elpida 200 mg tablets (Incepta Pharmaceuticals PLC, Bangladesh) were procured from the local market.

Chromatographic conditions

The analytical procedure was performed using an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm) thermostatted at 28 °C. This column was selected after brief screening against a shorter ODS C18 column and a C8 column. Sotagliflozin's moderate polarity favors a longer alkyl chain for adequate retention. The C8 column produced a retention time of approximately 3.2 minutes under identical mobile phase conditions, too close to the solvent front for reliable quantitation. The 250 mm C18 gave a retention time near 5 minutes with acceptable peak symmetry, so it was chosen for all further work. A UV detector was utilized for wavelength detection at 225 nm and LabSolutions software was employed for processing data. Acetonitrile and ammonium acetate in a 60:40 (v/v) ratio were used as mobile phase, adjusting pH to 3.0 by 0.05% (v/v) ortho-phosphoric acid. Throughout the whole analysis, a steady 1.0 mL min^{-1} flow rate was maintained. The mobile phase was degassed in an ultrasonic bath for approximately 15 minutes and then filtered through a 0.45 µm membrane filter. For each analysis, total run time was set to 10 minutes under environmental temperature conditions.

Method development

ATP and CAAs

The Analytical Target Profile (ATP) was defined as a stability-indicating RP-HPLC method capable of accurately and precisely quantifying sotagliflozin in bulk drug substance and tablet dosage forms in the presence of excipients and potential degradation products.[28,29] The method was intended to operate over a concentration range of 5–25 μg mL^{-1}, suitable for routine quality control applications. Acceptable method performance was predefined as accuracy within 98–102% recovery, precision with %RSD not exceeding 2%, and the ability to resolve sotagliflozin from excipients and degradation products. Chromatographic parameters such as tailing factor and theoretical plates were subsequently defined as Analytical Procedure Attributes (APAs) during the risk assessment phase, not as part of the ATP itself, in accordance with ICH Q14 guidance. These criteria were established to ensure reliable and reproducible performance throughout the method lifecycle. Based on this ATP, Retention time (Y1) as well as Tailing Factor (Y2) were selected as the CAAs for this study.

Risk assessment

Following the ATP definition, specific Analytical Procedure Attributes (APAs) were selected to evaluate method performance during development. These APAs include retention time, tailing factor and theoretical plates. Tailing factor (≤ 1.5) and theoretical plates (≥ 2000) were established as control strategy targets. These APAs were monitored throughout the Design of Experiments (DoE) to understand how they are influenced by the Critical Method Parameters (CMPs). Factors with the highest impact on the CAAs were identified through a risk assessment that combined a fishbone diagram (Figure 2) with a Failure Mode and Effects Analysis (FMEA).[30] This assessment identified percent acetonitrile (X1), flow rate (X2) and column temperature (X3) as high-risk CMPs selected for further investigation.

Single-factor screening

Before the DoE, five method parameters were screened using one-factor-at-a-time experiments. Acetonitrile was selected over methanol for lower backpressure (1600 psi vs. 2100 psi). A buffer concentration of 10 mM ammonium acetate gave the best signal-to-noise ratio. pH 3.0 was chosen after testing pH 2.5–4.2; tailing increased above pH 3.5, and pH below 2.8 raises column stability concerns. A 250 mm ODS C18 column was selected over a shorter C18 and a C8 column for best retention ($t_R \approx 5$ min) and theoretical plates (3865). Detection at 225 nm was chosen based on UV scan (λ_{max}). After fixing these five parameters, the remaining three variables (acetonitrile percentage, flow rate, and column temperature) were optimized using the Box-Behnken design.

Design of experiment (DoE)

A Box–Behnken experimental design was employed to evaluate the effects of critical method parameters and their interactions on the selected responses. This design was chosen due to its efficiency in requiring fewer experimental runs while adequately supporting quadratic model development. Additionally, the design avoids experimental conditions at the extreme corners of the factor space, which, based on preliminary trials, were associated with less stable chromatographic performance such as increased backpressure and suboptimal peak characteristics. Therefore, the Box–Behnken design was considered suitable for exploring the method operable region within practically relevant and controlled conditions. The design, comprising 17 experimental runs (Table I), was generated and analyzed using Design-Expert® (version 13) software to model all interactions between these Critical Method Parameters and the responses.

Table I. Applying Box-Behnken design for method optimization

Variable	Name	Unit	Type	Coded value		Actual value	
				Low	High	Low	High
A	Acetonitrile	%	Numeric	-1	1	50	60
B	Flow rate	mL/min	Numeric	-1	1	0.8	1.0
C	Column temperature	ºC	Numeric	-1	1	25	30

Runs	Factor A: Percent of acetonitrile	Factor B: Flow rate (mL/min)	Factor C: Column temperature	Response 1 RT (min)	Response 2 TF
1	55	0.8	30	5.411	0.859
2	60	0.9	30	4.811	0.724
3	50	0.9	30	5.445	0.853
4	55	0.9	27.5	5.501	0.862
5	55	1	25	5.401	0.745
6	50	0.8	27.5	6.012	0.934
7	55	0.9	27.5	5.441	0.851
8	55	0.9	27.5	5.488	0.840
9	55	1	30	4.744	0.711
10	50	1	27.5	5.449	0.857
11	55	0.9	27.5	5.469	0.853
12	55	0.9	27.5	5.478	0.858
13	60	0.9	25	5.332	0.801
14	60	0.8	27.5	5.350	0.852
15	60	1	27.5	4.820	0.741
16	50	0.9	25	5.984	0.975
17	55	0.8	25	6.044	0.957

Statistical analysis and optimization

Statistical evaluation and method optimization were conducted utilizing Design-Expert® software (version 13). The design generated the response values (Table I), which were subsequently analyzed using multiple linear regression analysis (MLRA). The relationship between the input variables and the responses was modeled using the statistically derived coded Equation (1).

$$Y = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X3 + \ldots\ldots\ldots\ldots + \beta_n Xn \quad (1)$$

Stock and working solution preparation

Sotagliflozin was dissolved in the mobile phase (acetonitrile:ammonium acetate buffer) to yield a primary stock solution of 1 mg mL^{-1}. A working solution of 100 µg mL^{-1} was prepared by appropriately diluting the

stock solution with the mobile phase. Serial dilution of the working solution with mobile phase yielded a calibration curve spanning 5-25 μg mL^{-1}.

Assay of marketed tablet

Twenty Elpida® tablets (200 mg each) were accurately weighed and finely powdered. A powder aliquot equivalent to 100 mg of sotagliflozin was treated with the mobile phase for dissolution and sonicated to make 100 mL of stock solution. After filtration, the stock was diluted 100-fold with mobile phase to yield 10 μg mL^{-1} solution for analysis.[31]

Validation of the method

Following method development and optimization, validation was performed in accordance with ICH Q2(R1) guidelines, assessing parameters such as accuracy, precision, robustness, and sensitivity.[32] All validation measurements, with the exception of system suitability testing, were performed as independent replicates. Each replicate represents a separate sample preparation from independent weighing, thereby capturing both sample preparation variability and measurement variability. System suitability was evaluated using repeated measures (six consecutive injections of a single standard solution) to assess instrument precision.

System Suitability

System suitability testing was conducted according to USP 40/NF 35 criteria to ensure adequate resolution of the liquid chromatographic system.[33] Repeated measures were performed, where a single standard solution (10 μg mL^{-1}) was injected six times consecutively. For the typical approach, parameters like peak area, RT, TF and TP were computed.

Specificity

Method specificity was determined to demonstrate selective quantification of sotagliflozin against potential interferents. This assessment was performed by chromatographically comparing a standard sotagliflozin solution with a placebo formulation. The analyte peak was well-defined and free from co-eluting components.

Accuracy

Method accuracy was evaluated through a standard addition recovery study. Known quantities of sotagliflozin standard were introduced into the sample matrix to achieve concentrations corresponding to 80%, 100%, and 120% of the target 10 μg mL^{-1} level. The percentage recovery of the analyte was determined at each of the three concentration levels. Three independent replicate preparations were analyzed (each injected once) to ensure reliability.

Precision

To assess repeatability within a single day, three different standard solutions of the drug (5, 15, and 25 μg mL^{-1}) were prepared, and each was analyzed three times under identical conditions. Intermediate precision was subsequently evaluated by repeating the analysis of the same concentration levels on three different days within one week.

Robustness

The robustness was assessed by introducing deliberate minor variations to the chromatographic conditions, evaluating its reliability and sensitivity under standard operating parameters.[34] Changes were made to the buffer pH to 2.8 and 3.2, the mobile phase composition to 58:42 and 62:38 (v/v), and the flow rate to 0.9 and 1.1 mL min^{-1} and column temperature to 23 ºC to 33 ºC. Robustness was evaluated based on the percent recovery and the associated %RSD values.

Ruggedness

Method ruggedness was assessed via an inter-analyst study, where two analysts independently prepared and analyzed six sample replicates (10 µg mL^{-1}) to determine reproducibility through % recovery and %RSD comparison.

Sensitivity

The detection and quantification limits were estimated using the signal-to-noise (S/N) approach and further calculated from the calibration curve statistics. Specifically, LOD and LOQ were derived from the slope of the calibration curve (S) along with the standard deviation (σ) of the y-intercept, as described in Equations (2) and (3).

$$LOD = (3.3 \times \sigma) / S \quad (2)$$

$$LOQ = (10 \times \sigma) / S \quad (3)$$

Forced degradation studies

For forced degradation studies, sotagliflozin in both Active Pharmaceutical Ingredient (API) and tablet form, was subjected to acid, alkali, neutral, oxidative, thermal, and photolytic stress. The resulting chromatograms were analyzed to assess peak purity and identify degradation products.[36,37]

Acidic and alkaline conditions

To induce acid degradation, 0.1 mL of stock solution was combined with 0.1 mL of 0.1 M HCl in mobile phase. For alkaline degradation, an equivalent volume of 0.1 M NaOH was used. The mixtures were heated at 40 °C and 60 °C for one hour, with a room temperature control included for each. After cooling, all samples were diluted to 10 µg mL^{-1} with mobile phase and analyzed by HPLC (20 µL injection volume).

Neutral and oxidative conditions

Neutral hydrolysis was induced by adding 0.1 mL of purified water to 0.1 mL of stock solution, followed by thermal stress at 40 °C and 60 °C. For oxidative degradation, 0.1 mL of stock solution was added with 0.1 mL of 3% hydrogen peroxide. All samples were then treated and analyzed under the same conditions as above.

Thermal degradation

Thermal degradation studies for both sotagliflozin API and the drug product involved diluting 0.1 mL stock aliquots to 10 µg mL^{-1}, followed by stress exposure at room temperature, 60°C, and 80 °C for 1-2 hours. After cooling and dilution to the final 10 µg mL^{-1} concentration with mobile phase, 20 µL injections were analyzed by HPLC.

Photolytic degradation

Photolytic degradation was performed using approximately 100 mg of solid API or powdered tablet, placed in lidded containers and exposed to near UV light (320–400 nm). Samples were withdrawn after 1, 3, and 5 days of exposure. For each interval, 10 mg of the exposed sample was diluted to prepare a 1 mg mL^{-1} stock solution. Protected control samples were analyzed concurrently. All solutions were sonicated, diluted to 10 µg mL^{-1} with mobile phase, and analyzed by HPLC using a 20 µL injection volume.

RESULTS AND DISCUSSION

The QbD framework is a systematic approach that uses statistical and risk-based methods to define a design space, establish controls, and enable continuous process improvement.[38] In order to strengthen method robustness and reduce failure, QbD is currently being widely used in a variety of sectors.[39] Using Analytical Quality by Design (AQbD) principals, this study established and implemented an analytical technique for the quantification of sotagliflozin.

Defining the Analytical Target Profile

The Analytical Target Profile (ATP) defined the required method performance characteristics, specifying its suitability for the analysis of sotagliflozin in both the API and tablet dosage form (Table II). A reverse-phase HPLC method was adopted based on the analyte's polarity and chemical characteristics. The ATP also guided sample preparation, including selection of appropriate diluents according to solubility and the solid nature of the samples. This defined ATP provided the basis for the subsequent method development and risk assessment steps.

Table II. Analytical target profile for sotagliflozin RP-HPLC method

ATP Element	Target / Criterion	Justification
Sample matrix	Pure API and tablet excipients	Method must resolve drug from all formulation components
Interferences to be resolved	Degradation products from forced degradation	Stability-indicating requirement per ICH Q1A(R2)
Concentration range	5–25 μg mL^{-1} (50–250% of nominal 10 μg mL^{-1} test concentration)	Covers expected potency variations and stability study levels
Accuracy acceptance limit	Mean recovery 98.0–102.0% at 80%, 100%, and 120% levels	ICH Q2(R1) guideline for assay of drug substance or product
Precision acceptance limit	%RSD ≤ 2.0% for repeatability and intermediate precision	Standard industry practice for HPLC assay
Method application	Assay of API and finished product	Routine quality control and stability testing

Risk assessment and Critical Method Parameter (CMP) selection

A risk assessment prioritized method parameters impacting the CAAs: retention time (Y1) and tailing factor (Y2). Parameters were evaluated using a fishbone diagram and ranked based on the severity of their impact and probability of occurrence (Table III). Percent ACN, flow rate, and column temperature were defined as high-risk and selected for experimental design, while low-risk parameters were fixed.

Table III. Risk assessment and prioritization of method parameters using FMEA scoring

Method Parameter	Effect on CAAs	Severity (S) (1-10)	Occurrence (O) (1-10)	Detection (D) (1-10)	RPN (S×O×D)	Risk Priority	Remarks
Percent acetonitrile	Affects retention and peak shape	9	8	5	360	High	Selected for DoE
Flow rate	Affects retention time and backpressure	8	7	4	224	High	Selected for DoE
Column temperature	Affects retention and efficiency	7	6	6	252	High	Selected for DoE
Buffer pH	Affects ionization and peak shape	7	4	4	112	Medium	Optimized separately, fixed before DoE
Stationary phase type	Determines selectivity and retention	8	3	3	72	Medium	Fixed based on literature

(continued on next page)

Table III. Risk assessment and prioritization of method parameters using FMEA scoring (continued)

Method Parameter	Effect on CAAs	Severity (S) (1-10)	Occurrence (O) (1-10)	Detection (D) (1-10)	RPN (S×O×D)	Risk Priority	Remarks
Detection wavelength	Affects sensitivity but not separation	6	3	2	36	Low	Fixed at λ_{max}
Injection volume	Affects peak shape at high volumes	4	4	3	48	Low	Fixed at 20 µL

Scoring basis: Severity (1 = minimal impact on results, 10 = method fails completely); Occurrence (1 = very unlikely to vary, 10 = varies frequently); Detection (1 = easily detected before reporting, 10 = not detectable). Risk Priority Number (RPN) ≥ 50 considered high/medium risk requiring control.

Method development

RP-HPLC method development was structured around the AQbD framework, beginning with the interpretation of the Analytical Target Profile. This study intended to establish and evaluate a quick, sensitive AQbD-based methodology for sotagliflozin to overcome the inefficiencies of traditional one-factor-at-a-time optimization which can be expensive and delayed.[40] Method development focused on mobile phase selection, whereas the organic modifier acetonitrile was chosen after systematic solvent evaluation. The aqueous phase was optimized by testing various ammonium acetate buffer concentrations across a pH range of 2.5–4.2. Final method conditions employed an acetonitrile-ammonium acetate buffer combination at an optimized pH.

Data analysis

Table I displays the outcomes of several experimental runs. The best-fitting models were identified by performing analysis of variance (ANOVA) on the collected responses, as shown in Table IV. These models were identified to be significant ($p < 0.05$). Figure 3 shows the corresponding response surface diagrams.

Table IV. ANOVA results and model fit statistics for the CQAs: retention time (Y1) and tailing factor (Y2)

Responses	Source	SS	DF	*F*-value	*p*-value	Remarks
Y1 (Retention Time)	Linear Model	2.24	3	274.38	< 0.0001	Significant
	Residual	0.0354	13			
	Cor Total	2.28	16			
Y2 (Tailing Factor)	Linear Model	0.0826	3	33.63	< 0.0001	Significant
	Residual	0.0106	13			
	Cor Total	0.0933	16			

Fit statistics							
Source	**Standard Deviation**	**Mean**	**Coefficient of Variation (%)**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**Adequate Precision**
R1	0.0522	5.42	0.9625	0.9845	0.9809	0.9743	49.1767
R2	0.0286	0.8396	3.41	0.8859	0.8595	0.7730	18.8943

Effect of CMPs on CAAs

Several experimental runs that were performed using a Box Behnken design to assess the linear influences and interaction of critical method parameters on critical analytical attributes are shown in Table IV. The use of a Box-Behnken experimental design enabled systematic exploration of these variables and their interactions, resulting in the development of a statistically significant response model. This approach allowed the establishment of a Method Operable Design Region (MODR) in which chromatographic performance remained predictable and within predefined acceptance limits. The MODR was defined using a 95% prediction interval approach. For retention time, which was to be minimized, the MODR represents the region where the upper prediction bound is as low as possible while still maintaining acceptable chromatographic performance. For tailing factor, which was targeted at 1.0, the MODR represents the region where the 95% prediction interval falls within 0.8–1.2. The verified MODR within the tested experimental range (50–60% acetonitrile, 0.8–1.0 mL/min flow rate, 25–30 °C column temperature) is shown in Figure 3E as the shaded overlapping region where both criteria are simultaneously met.

Retention time (Y1)

The retention time as outlined in Table I for the optimized quantification of sotagliflozin ranged between 4.744 and 6.044 minutes. This retention time was primarily influenced by the percent acetonitrile, flow rate and column temperature. Figure 3 illustrates how these variables affected retention time.

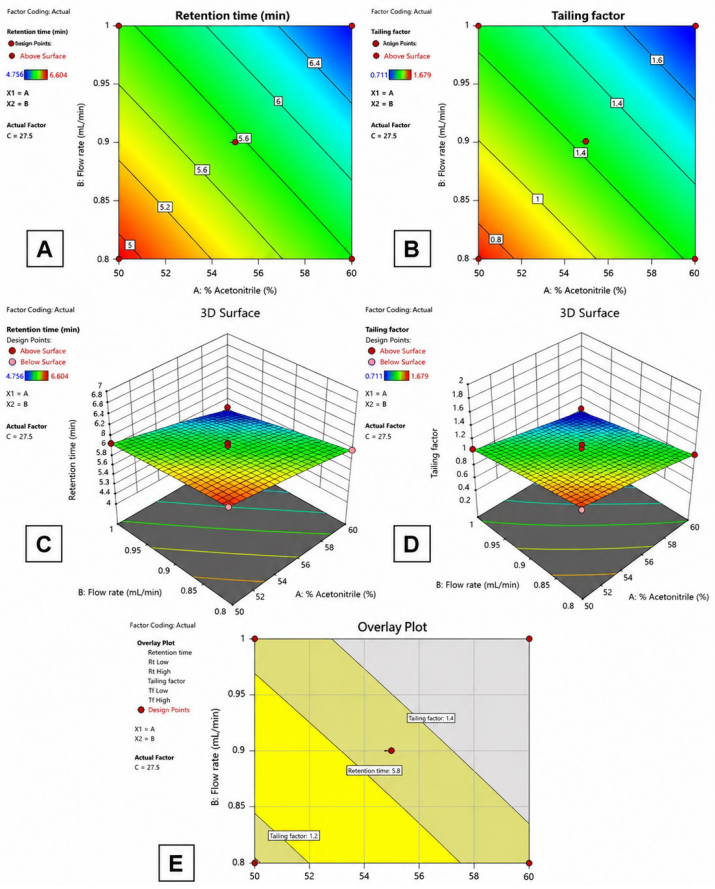

Figure 3. Response surface plots depicting the influence of key method parameters on chromatographic performance. (A) Contour representation of retention time (R1); (B) Contour representation of tailing factor (R2); (C) Three-dimensional surface plot showing retention time (R1); (D) Three-dimensional surface plot illustrating tailing factor (R2); and (E) Overlaid contour plot showing the Method Operable Design Region (MODR) where both retention time and tailing factor simultaneously meet acceptance criteria.

An increase in any independent variable shortened the retention time (Y1), demonstrating a negative correlation, as shown in Equation (4).

$$Y1(\text{Retention time}) = 14.90035 - 0.064425X_1 - 3.00375X_2 - 0.11750X_3 \quad (4)$$

Tailing factor (Y2)

As presented in Table I, the tailing factor (Y2) ranged from 0.711 to 0.975. The independent variables X_1, X_2, and X_3 demonstrated a statistically significant effect on the response (p value < 0.05). Figure 3 depicts the negative influence of all independent variables on the tailing factor (Y2), a relationship modeled by Linear Equation (5).

$$Y2(\text{Tailing factor}) = 2.6001 - 0.0125X_1 - 0.6850X_2 - 0.0166X_3 \quad (5)$$

The multi-response optimization successfully identified chromatographic conditions that minimized retention time and brought the tailing factor close to the ideal value of 1.0 while maintaining all independent variables within their operational ranges (Table V). The optimal solution (desirability = 1.000) comprised 60% acetonitrile, 1.0 mL min^{-1} flow rate and 28 °C column temperature. Experimental validation demonstrated acceptable agreement with model predictions (≤2.0% error), with all response parameters complying with predefined acceptance criteria (Table V).

Using the established linear models (Equations 4 and 5), the Method Operable Design Region (MODR) representing the robust multidimensional design space where critical analytical attributes (RT and TF) stay acceptable was defined. This MODR was derived from overlaid contour plots (Figure 3).

Table V. Predicted response optimization with error analysis

Independent Variables	Criteria	Responses	Criteria
% acetonitrile	In range	R1 (RT)	Minimize
Flow rate	In range	R2 (TF)	Target = 1.0
Column temperature	In range		

Method	% of acetonitrile	Flow rate (mL min^{-1})	Column temperature	Response 1 (RT)	Response 2 (TF)
Predicted values	60	1.0	28	4.737	0.701
Experimental values	60	1.0	28	4.706	0.698
Predicted errors (%)*				0.654%	0.427%

* Predicted errors (%) = [(Experimental value - Predicted value)/predicted value] × 100%

Prediction error was evaluated using three independent replicate experiments at the optimized conditions. The reported experimental values in Table V are the means of these three replicates. The %RSD across replicates was 0.45% for retention time and 1.09% for tailing factor.

Method validation

The method underwent validation to establish its suitability for routine analysis. The method met all system suitability requirements, as evidenced by the high precision of retention time, peak area, TF and TP count from six replicate standard injections (Table VI). The method exhibited excellent specificity, as evidenced by a well-resolved and symmetric peak for the analyte with no interfering signals from blank matrices at

the corresponding retention time (Figure 4). Excellent linearity (R^2 = 0.9991) was demonstrated over the concentration range of 5–25 µg mL^{-1}, confirming a direct proportional relationship between concentration and detector response. Acceptable accuracy was demonstrated across the tested concentration range, with mean recoveries of 99.02–100.02% and %RSD values not exceeding 0.73% (Table VI). The method also demonstrated high precision, as both intra-day and inter-day variations resulted in %RSD values within acceptable limits, indicating acceptable repeatability and intermediate precision.

Table VI. Method validation results for sotagliflozin by RP-HPLC

Parameter	**Average ± %RSD**	**Permissible ranges**	**Type of study**	**Sotagliflozin (µg mL^{-1})**	**Added concentration (%)**	***Mean % Recovery**	**%RSD**
System suitability			**Accuracy**				
Peak area	2095789.53 ± 1.25		%RSD ≤ 2	10	80	99.02	0.92
Tailing factor	0.713 ± 1.09%		≤ 1.5	10	100	101.29	0.15
Retention time	4.745 ± 0.45%		%RSD ≤ 0.5	10	120	100.46	0.06
Theoretical plate	3865.74 ± 0.96%		≥ 2000				
Parameter	**Interday precision**				**Intraday precision**		
Sl. No.	**Sample conc. taken (µg mL^{-1})**	***Mean % Recovery**	**%RSD**		**Sample conc. taken (µg mL^{-1})**	***Mean % Recovery**	**%RSD**
1	5	99.51	0.51		5	101.45	0.30
2	15	100.87	0.87		15	101.29	0.73
3	25	98.84	0.95		25	100.02	0.49
Parameter	**Type**					***Mean % Recovery**	**%RSD**
Ruggedness	Analyst-1					99.57	0.55
	Analyst-2					100.06	0.67
Test	**Parameters**		**Variations**	**Added amount (µg mL^{-1})**		***Mean % Recovery**	**%RSD**
Robustness	Flow rate (mL min^{-1})		0.9	10		101.42	0.99
			1.0	10		101.01	0.45
			1.1	*10*		*100.46*	*0.37*
	Mobile phase (Acetonitrile:Buffer)		58:42	10		99.46	0.85
			60:40	10		99.74	0.95
			62:38	*10*		*98.85*	*0.76*
	Mobile phase (pH)		2.8	10		100.78	0.79
			3.0	10		99.41	1.11
			3.2	10		101.52	0.87
	Column temperature		*23*	*10*		*101.75*	*0.85*
			28	10		98.10	1.07
			33	*10*		*100.74*	*1.08*

*Data represent the mean of three determinations (n = 3).
Conditions shown in italics (flow rate 1.1 mL/min, mobile phase 62:38, temperature 23 °C and 33 °C) are outside the original Box-Behnken experimental range. These tests were performed only to assess method tolerance and do not form part of the claimed MODR.

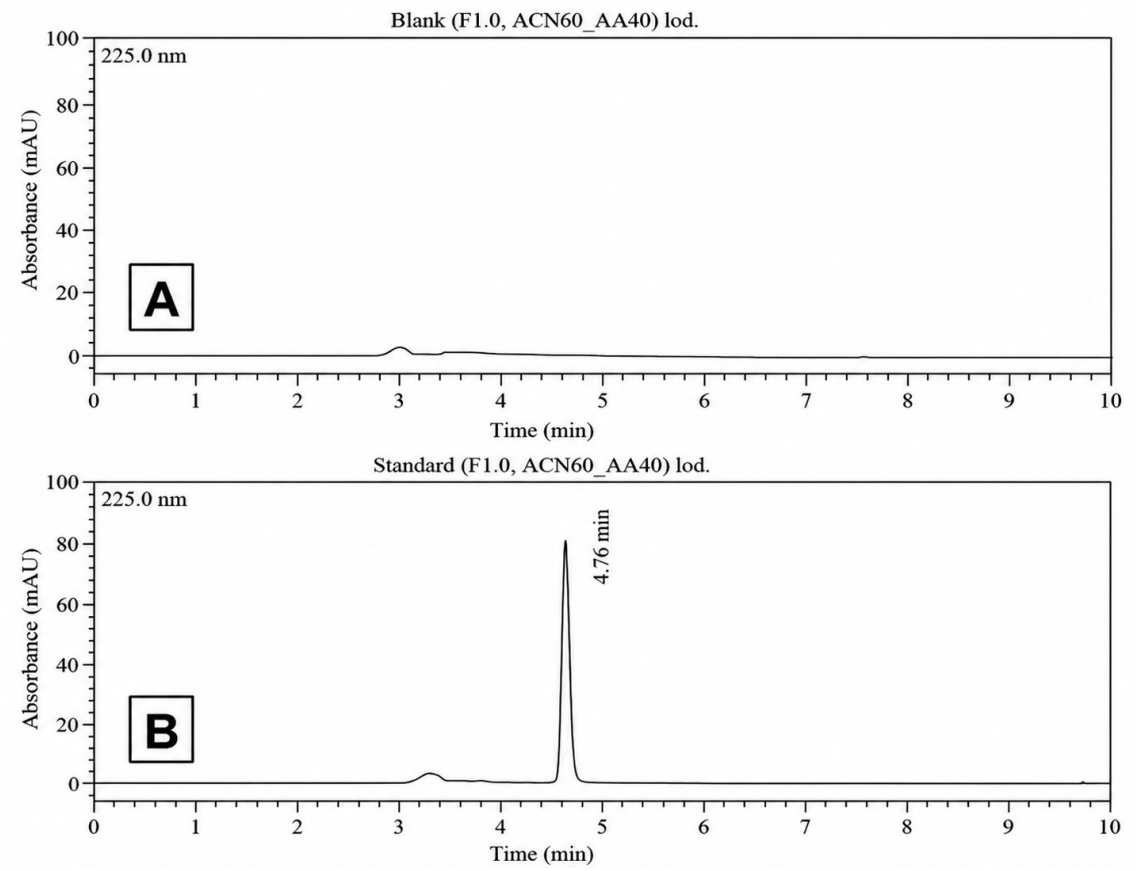

Figure 4. Chromatograms demonstrating method specificity: (A) blank and (B) 10 µg mL^{-1} sotagliflozin standard.

Sensitivity was established with a LOD of 0.12 µg mL^{-1} and a LOQ of 0.38 µg mL^{-1}, underscoring the method's capability to analyze trace levels of the analyte (Figure 5). Ruggedness, evaluated through analysis by two different analysts, showed minimal variability (%RSD 0.55%–0.67%), confirming the method's consistency across different operators. Furthermore, robustness testing under intended variations in % ACN composition, pH, column temperature and flow rate resulted in only minor shifts in chromatographic behavior while maintaining recovery values with %RSD between 0.37% and 1.11%, attesting to the method's resilience under moderate operational changes.

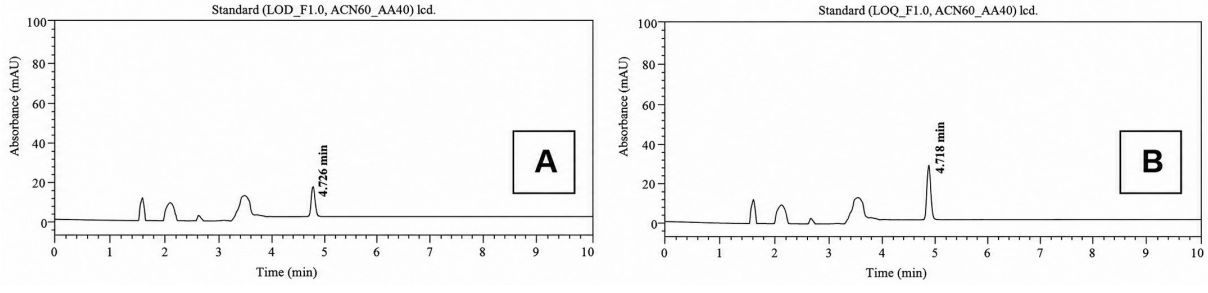

Figure 5. Representative chromatograms demonstrating the (A) LOD and (B) LOQ.

Assay of marketed tablet formulation

The validated method was applied to quantify sotagliflozin in Elpida® 200 mg tablets. The mean recovery was 99.43% of the labeled claim (Table VII), with a relative standard deviation of 1.24% (n = 3). No interfering peaks from tablet excipients were observed at the retention time (4.702 min) of sotagliflozin (Figure 6).

Table VII. Analysis of sotagliflozin marketed tablet

Marketed formulation containing sotagliflozin	Amount added (μg mL^{-1})	Amount recovered (μg mL^{-1})	*Mean % Recovery	%RSD
Elpida® 200 mg tablets	10.00	9.96	99.43	1.24%

* Data represent the mean of three determinations (n = 3).

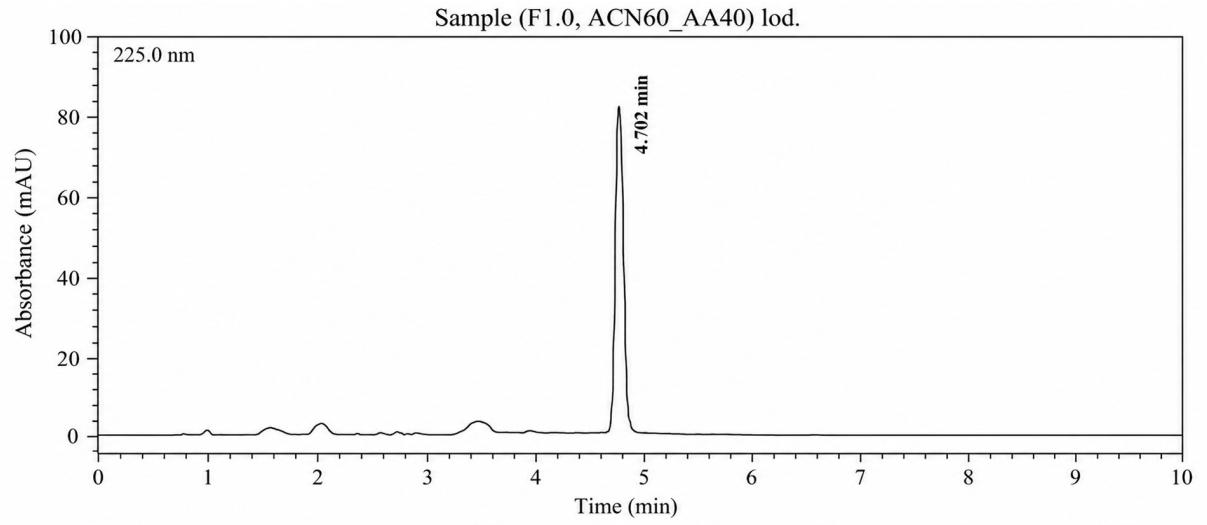

Figure 6. Representative chromatogram of the marketed sotagliflozin tablet formulation.

Forced degradation

The method demonstrated its stability-indicating capability by effectively separating sotagliflozin from its degradation products in all conditions, with peak purity indices > 0.98, confirming the homogeneity of the analyte peak. Sotagliflozin demonstrated significant degradation under acidic, alkaline and thermal conditions. In acidic medium (0.1 M HCl), the drug showed 26.45% degradation after 1 hour at 60 °C, while alkaline conditions (0.1 M NaOH) resulted in more substantial degradation (29.29% after 1 hour at 60 °C). Under thermal stress at 60 °C, the drug substance showed significant degradation, reaching 23.58% after 1 h (Table VIII).

Table VIII. Forced degradation studies of sotagliflozin

Degradation	**Acid (HCl 0.1 N)**			**Base (NaOH 0.1 N)**		
Condition	RT, 1 h	40 °C, 1 h	60 °C, 1 h	RT, 1 h	40 °C, 1 h	60 °C,1 h
Standard	12.36	18.28	26.45	11.10	21.45	29.29
Sample	8.67	13.31	19.56	9.15	18.28	26.91

(continued on next page)

Table VIII. Forced degradation studies of sotagliflozin (continued)

Degradation	**Neutral (Hydrolytic)**			**Dry heat**		
Condition	RT, 1 h	40 °C, 1 h	60 °C, 1 h	RT, 1 h	40 °C, 1 h	60 °C, 1 h
Standard	5.10	8.36	14.47	11.78	14.46	23.58
Sample	3.45	7.48	11.52	4.78	8.69	19.87
Degradation	**Oxidative (H_2O_2 3%)**			**UV Light**		
Condition	RT, 1 h	40 °C, 1 h	60 °C, 1 h	1 day	3 days	5 days
Standard	4.03	9.37	12.37	2.56	4.21	6.14
Sample	3.33	7.94	10.4	1.89	3.59	5.49

The drug exhibited moderate stability under hydrolytic stress, showing 14.47% degradation at temperatures of 60 °C for 1 h. Oxidative stress with 3% hydrogen peroxide caused 12.37% degradation after 1 hour at 60 °C (Table VIII).

Photolytic degradation studies revealed that sotagliflozin demonstrated substantial photostability up to 24 hours of direct UV light exposure. However, extended exposure resulted in decomposition, with maximum degradation reaching 6.14% for the drug substance after 120 hours of continuous exposure (Figure 7). The formulated product consistently showed lower degradation levels than the pure drug under all stress conditions, an observation that may warrant further investigation into possible excipient interactions.

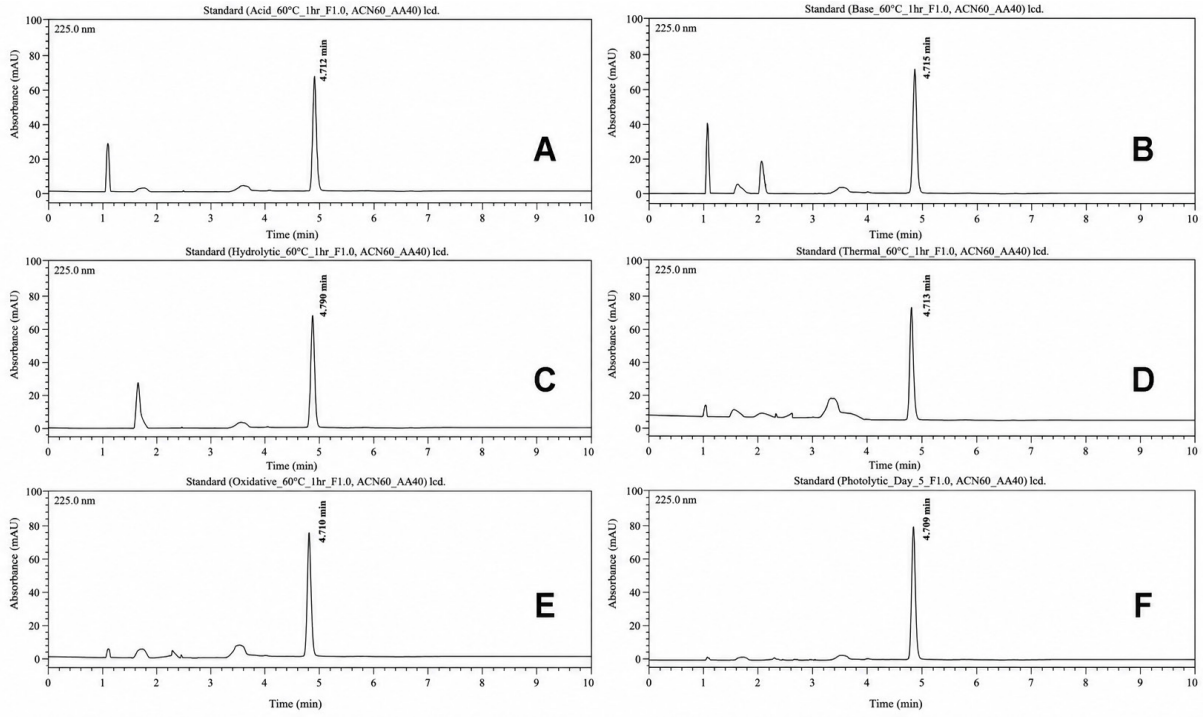

Figure 7. Forced degradation chromatographic profiles of sotagliflozin: (A) Acidic, (B) Alkaline, (C) Neutral, (D) Thermal, (E) Oxidative, and (F) Photolytic conditions.

Chromatograms under each stress condition demonstrated clear separation between sotagliflozin and its degradation peaks. Chromatographic analysis showed a single, pure peak for sotagliflozin in all stressed

samples, proving the method's capability to specifically quantify the drug in the presence of its degradants. Sotagliflozin showed pronounced degradation under hydrolytic conditions in both acidic and alkaline media, as well as under elevated temperature, with degradation levels ranging from approximately 23–29%. Comparatively lower degradation was observed during oxidative, neutral and photolytic stress. Importantly, under all applied stress conditions, the developed method was able to resolve the intact analyte from its degradation products which confirmed the specificity of the chromatographic conditions. Notably, the tablet formulation exhibited greater resistance to stress conditions than the pure drug. This observation suggested a stabilizing influence of excipients, which is valuable information for formulation scientists.

Several analytical methods for sotagliflozin have been reported, including UV spectrophotometry, conventional RP-HPLC, UPLC, and LC-MS/MS.[9-13] For instance, Karanam *et al.* developed a UV/RP-HPLC method using one-factor-at-a-time optimization, achieving good linearity and recovery but with limited insight into parameter interactions and robustness.[10] Similarly, Sivakumar *et al.* described a stability-indicating UPLC method offering fast analysis yet lacking a risk-based design space,[11] while Chatterjee *et al.* reported a UPLC-PDA method with very short retention (t_R = 0.516 min), raising concerns over selectivity and reproducibility.[12] In contrast, the present AQbD-based RP-HPLC method offers unique and substantial advantages: approximately 20% shorter development time through efficient Design of Experiments (Table IX), a defined MODR providing enhanced robustness (e.g., tolerance to ±2% acetonitrile variation with <1.11% RSD in recovery), and the first demonstration of excipient-mediated stabilization in tablet formulations (19–26% less degradation compared to pure API under stress conditions). It is worth noting that Sivakumar *et al.* provided LC-MS/MS data for degradant identification, a dimension not explored in the current study.[11] This represents a limitation of our work and an avenue for future investigation. Another limitation of our study is that known impurities were not included in the experimental design as potential interferents. Resolution from impurities would have been a valuable additional critical analytical attribute for a comprehensive stability-indicating method design. Future work incorporating commercially available impurity reference standards into the DoE framework would provide a more complete design space definition. Additionally, LC-MS analysis of stressed samples would provide definitive confirmation of peak purity beyond the capabilities of diode array detection.

Table IX. Comparison of the developed AQbD method with reported chromatographic methods for sotagliflozin

Parameter	This work	Karanam *et al.* [10]	Sivakumar *et al.* [11]	Chatterjee *et al.* [12]
Technique	RP-HPLC (AQbD)	RP-HPLC (OFAT)	UPLC	UPLC-PDA
Mobile Phase	ACN:Ammonium acetate (60:40)	ACN:Water	Not detailed	ACN:Water
Run time (min)	10	~6	~5	~1.5
Retention time (min)	~4.7	~3.7	~0.761	0.516
Linearity range (µg mL^{-1})	5-25	10-50	4-60	10-50
LOD (µg mL^{-1})	0.12	0.03	0.12	0.05
LOQ (µg mL^{-1})	0.38	0.12	0.4	0.1
Robustness tested	Yes (DoE-based)	Limited	Limited	No
Design space defined	Yes (MODR)	No	No	No
Stability-indicating	Yes	No	Yes	No
Degradation Pathway Analysis	No	No	Yes (LC-MS)	No

CONCLUSIONS

Employing an AQbD framework, a new stability-indicating RP-HPLC method was developed for quantifying sotagliflozin in API and pharmaceutical formulations. Initial risk assessment, facilitated by a fishbone diagram identified CMPs with significant impact on the predefined Critical Analytical Attributes. A Box-Behnken design was employed for screening and optimizing these CMPs to meet the Analytical Target Profile. The optimization process defined a set of robust and reliable chromatographic conditions that simultaneously provided a short analysis time and acceptable peak shape. However, a limitation of our AQbD workflow is that the hybrid OFAT-plus-DoE approach does not evaluate interactions between fixed parameters (pH, stationary phase) and optimized CMPs. A full sequential DoE (screening followed by optimization) would provide a more complete understanding of the analytical procedure.

The method validation confirmed excellent performance, demonstrating high linearity, accuracy, precision, robustness, and specificity. Stress testing performed per ICH Q1A(R2) validated the method as stability-indicating. The results showed pronounced degradation in acidic environments, in contrast to the minor decomposition observed under neutral, oxidative, and photolytic stress. While the method successfully separated sotagliflozin from its degradation products, future work will focus on the structural identification of the major degradants using advanced techniques like LC-MS/MS to elucidate the complete degradation pathways. This risk-based AQbD strategy proves the procedure is highly appropriate for routine quality evaluation, and shows promising potential for application in routine quality control and stability testing.

Acknowledgements

The authors declare that no funding was received for this work.

Conflicts of interest

The authors declare no conflicts of interest.

Use of artificial intelligence tools

The authors declare that they used Deepseek for language editing. The authors take full responsibility for the accuracy, integrity, and originality of the article's content and confirm that no AI tool was used to generate novel scientific ideas, analyze data independently, or replace the critical role of the authors.

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