



Development and Validation of a Robust RP-HPLC Method for the Quantification of Empagliflozin, a Selective SGLT-2 Inhibitor, Using Quality-by-Design Approach

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ABSTRACT:

Empagliflozin is a selective sodium-glucose cotransporter-2 (SGLT-2) inhibitor for which reliable quantitative chromatographic analysis is required during pharmaceutical quality control. Quality by Design (AQbD) strategy was used to organize the development of a reversed-phase high-performance liquid chromatographic (RP-HPLC) method for empagliflozin. Preliminary chromatographic investigations supported use of a C18 stationary phase, phosphate buffer–acetonitrile mobile phase, PDA detection and a short analysis time. Detection wavelength and flow rate were evaluated as experimental factors in a two-factor, three-level, nine-run design, while peak area, theoretical plates, asymmetry and retention time were treated as analytical responses. The supplied nine-run dataset covered wavelengths of 224, 227 and 230 nm and flow rates of 0.9, 1.0 and 1.1 mL/min. Peak area ranged from 19,134 to 19,354; theoretical plates from 7,388 to 7,632; asymmetry from 1.24 to 1.31; and retention time from 3.13 to 3.21 min. Reported ANOVA showed significant models for peak area ($F=6.86$, $p=0.0319$), theoretical plates ($F=12.50$, $p=0.0095$) and retention time ($F=65.0$, $p<0.0001$); the asymmetry model F -value was 15.83. The published optimized procedure used a Discovery C18 column with 0.05 M potassium dihydrogen phosphate buffer (pH 3.0): acetonitrile (40:60, v/v), 1.0 mL/min, PDA detection at 228 nm, 40°C and 20 μ L injection volume, with reported empagliflozin retention at 2.31 min in a 5-min run. Validation data included a 20–160 μ g/mL linearity range, intraday and interday precision of 1.14% and 1.12% RSD, overall recovery of 99.80%, LOD of 0.312 μ g/mL and LOQ of 0.662 μ g/mL. Standard and test solutions were stable for 6 h. The method provides a rapid and systematic QbD-oriented framework for routine empagliflozin quantification; however, the experimental wavelength/final-method discrepancy should be reconciled against the original laboratory and Design-Expert records before submission.

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1. INTRODUCTION:

Empagliflozin is an orally active sodium-glucose cotransporter-2 (SGLT-2) inhibitor that lowers blood glucose by reducing renal glucose reabsorption and increasing urinary glucose excretion.^{1,2} Its pharmaceutical analysis therefore requires a quantitative method capable of delivering reproducible detector response, acceptable peak shape and consistent retention under routine laboratory conditions.

Several analytical procedures have been reported for empagliflozin. Hanif et al. described an HPLC method for pharmaceutical raw material and dosage-form analysis with a broad analytical response and satisfactory accuracy and precision.³ More recent work has addressed stability-indicating chromatographic determination, including separation of empagliflozin from degradation products and synthetic impurities.^{4,5}

Conventional chromatographic method development may rely on sequential experimentation and can provide limited information about interactions between method variables. Analytical Quality by Design (QbD) integrates an analytical target profile, risk-based selection of critical method parameters, Design of Experiments (DoE), statistical modeling and establishment of an appropriate operating region.^{6,7} Recent QbD literature emphasizes that structured factor–response assessment can improve method understanding and robustness.

ICH Q14 provides a science- and risk-based framework for analytical procedure development, whereas ICH Q2(R2) provides the current framework for validation of analytical procedures.^{8,9} These principles support a lifecycle-oriented approach in which method development knowledge is linked to validation and control of critical analytical performance characteristics.

The present manuscript reorganizes the supplied empagliflozin development and validation information using the section structure of the provided ivabradine hydrochloride QbD manuscript. The objective was to evaluate detection wavelength and flow rate against peak area, column efficiency, peak symmetry and retention time, and to present the available validation and solution-stability data in a journal-oriented format. A key source-data issue is retained explicitly: the supplied progress report uses 224/227/230 nm and 0.9/1.0/1.1 mL/min, whereas the published empagliflozin article reports 226/228/230 nm and 0.8/1.0/1.2 mL/min for its factorial design.¹⁰ This discrepancy must be resolved before submission.



Figure 1. Chemical structure of empagliflozin

2. MATERIALS AND METHODS:

2.1 Materials

Empagliflozin study material was reported in the supplied documents as empagliflozin/empagliflozin HCl and was obtained as a gift sample from Lupin Pharmaceuticals Ltd., Pune, Maharashtra, India. Analytical-grade reagents were used. Potassium dihydrogen phosphate and acetonitrile were used for preparation of the mobile phase and analytical solutions.¹⁰

2.2 Instrumentation

Chromatographic analysis was performed using an Agilent 1260 Infinity HPLC system equipped with an autosampler and PDA detector. The source publication reports use of a Discovery C18 column (250 × 4.6 mm, 5 μm), with chromatographic data acquisition and processing through OpenLab EZChrom.^{1,10}

2.3 Chromatographic conditions

The reported final chromatographic procedure used a Discovery C18 column (250 × 4.6 mm, 5 μm), 0.05 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 and acetonitrile in a 40:60 (v/v) ratio, a flow rate of 1.0 mL/min, PDA detection at 228 nm, column temperature of 40°C, 20 μL injection volume and a 5-min run time. Empagliflozin was reported to elute at 2.31 min.^{10,1}

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Table 1. Optimized chromatographic conditions reported in the supplied source article.

Parameter	Optimized condition
Column	Discovery C18, 250 × 4.6 mm, 5 µm
Mobile phase	0.05 M KH ₂ PO ₄ buffer pH 3.0 : acetonitrile (40:60, v/v)
Flow rate	1.0 mL/min
Detection	PDA, 228 nm
Column temperature	40°C
Injection volume	20 µL
Run time	5 min
Reported retention time	2.31 min

2.4 Detection wavelength

PDA detection was reported at 228 nm in the final published procedure.^{10,21} The progress-report DoE matrix, however, lists 224, 227 and 230 nm as the investigated wavelength levels. The two records should be reconciled with the original instrument method before the final manuscript is submitted.¹

2.5 Column selection

A reversed-phase C18 stationary phase was selected during preliminary chromatographic investigations on the basis of chromatographic performance and system-suitability characteristics.^{10,1}

2.6 Mobile-phase selection

Preliminary chromatographic trials evaluated buffer/organic-solvent combinations. The reported final composition was 0.05 M potassium dihydrogen phosphate buffer at pH 3.0 and acetonitrile in a 40:60 (v/v) ratio.^{10,1}

2.7 Standard preparation

A 1000 µg/mL stock solution was prepared by accurately weighing 10 mg of empagliflozin into a 10-mL volumetric flask and diluting to volume with the selected diluent. Working solutions were prepared by appropriate dilution with the mobile phase. Fresh stock and working solutions were reported to be prepared daily. The validated calibration range was 20–160 µg/mL.^{10,1}

2.8 Sample preparation

The supplied source article states that the procedure was applicable to bulk drug and pharmaceutical dosage forms; however, the complete formulation-specific weighing, extraction and dilution sequence is not available in the supplied manuscript extract. The original laboratory record should therefore be used to insert the exact tablet sample-preparation procedure before submission.^{10,1}

2.9 Statistical analysis

The nine experimental combinations were evaluated using the reported DoE/ANOVA results. Peak area (R1), theoretical plates (R2), asymmetry (R3) and retention time (R4) were treated as analytical responses. Model significance was interpreted at $p < 0.05$. The supplied report does not contain complete regression equations, R^2 , adjusted R^2 , predicted R^2 , lack-of-fit statistics or desirability values; these should not be reconstructed without the original Design-Expert output.^{6,8,9,10,1}

3. QbD-BASED METHOD DEVELOPMENT

3.1 Analytical Target Profile

The analytical target was a rapid, reproducible and sufficiently robust RP-HPLC procedure for quantitative determination of empagliflozin, providing adequate detector response, satisfactory column efficiency, acceptable peak symmetry and short retention time for routine quality-control analysis. The monitored responses were peak area, theoretical plates, asymmetry and retention time.

3.2 Risk assessment

The supplied progress report does not provide a formal numerical risk-assessment matrix or risk-priority-number calculation. Accordingly, no unsupported numerical risk scores have been introduced. Detection wavelength and flow rate are treated as experimentally investigated method variables, and their effects are discussed using the available ANOVA findings.

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3.3 Critical Method Parameters

Detection wavelength (A) and flow rate (B) were selected as the independent chromatographic variables in the supplied progress-report dataset. Wavelength was studied at 224, 227 and 230 nm, while flow rate was studied at 0.9, 1.0 and 1.1 mL/min.

3.4 Critical Analytical Responses

Peak area (R1), theoretical plate count (R2), asymmetry (R3) and retention time (R4) were monitored. Together, these responses represent detector response, column efficiency, peak shape and chromatographic speed, respectively.

3.5 Design of Experiments

A two-factor, three-level experimental design comprising nine experimental combinations (B1–B9) was used in the supplied progress report. The observed data are reproduced without alteration.

3.6 Experimental design

Table 2. Nine-run QbD experimental matrix and observed chromatographic responses.

Run	Wavelength (nm)	Flow rate (mL/min)	Peak area	Theoretical plates	Asymmetry	Retention time (min)
B1	224	0.9	19354	7632	1.31	3.16
B2	224	1.0	19223	7554	1.26	3.2
B3	224	1.1	19134	7526	1.24	3.16
B4	227	0.9	19168	7446	1.27	3.18
B5	227	1.0	19208	7506	1.25	3.15
B6	227	1.1	19247	7535	1.31	3.13
B7	230	0.9	19183	7402	1.3	3.21
B8	230	1.0	19226	7388	1.27	3.19
B9	230	1.1	19318	7565	1.31	3.14

4. RESULTS AND DISCUSSION:

4.1 Preliminary method development

Preliminary chromatographic investigations supported selection of a C18 column and phosphate buffer–acetonitrile mobile phase. The final procedure reported in the supplied publication used 0.05 M potassium dihydrogen phosphate buffer at pH 3.0:acetonitrile (40:60, v/v), 1.0 mL/min flow, 40°C column temperature and 228 nm PDA detection. Under the reported final conditions, empagliflozin eluted at 2.31 min in a 5-min run.¹⁰

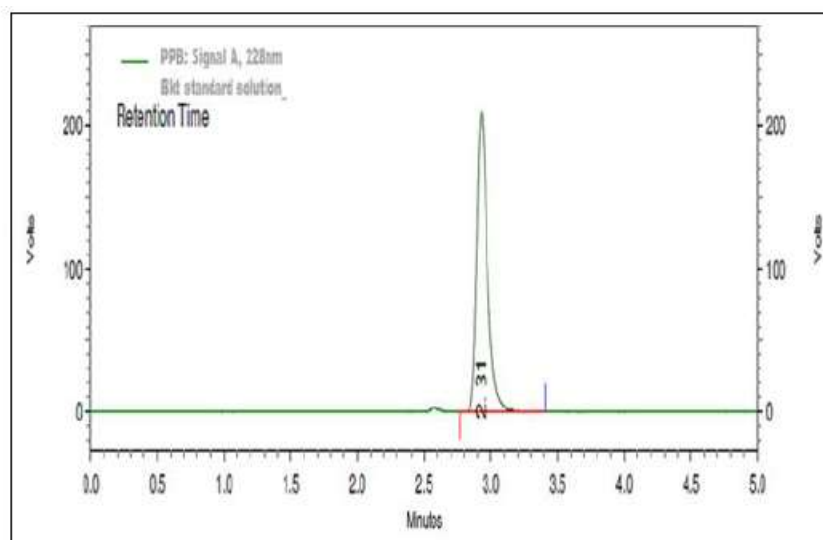


Figure 1. Observed response profile across the nine experimental runs. The plot summarizes peak-area and retention-time behavior

4.2 Experimental design results

The nine-run dataset showed relatively narrow response ranges. Peak area ranged from 19,134 to 19,354; theoretical plates from 7,388 to 7,632; asymmetry from 1.24 to 1.31; and retention time from 3.13 to 3.21 min. The calculated mean responses were 19,229 peak-area units, 7,506 theoretical plates, 1.28 asymmetry and 3.169

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min retention time.

4.3 Effect on peak area

Peak area varied from 19,134 to 19,354. The highest response occurred at 224 nm and 0.9 mL/min (B1), whereas the lowest occurred at 224 nm and 1.1 mL/min (B3). The limited magnitude of variation indicates comparatively stable detector response within the investigated factor region. The reported ANOVA model was significant ($F=6.86$, $p=0.0319$), and the wavelength $\times$ flow-rate interaction was reported as significant ($p=0.0063$).

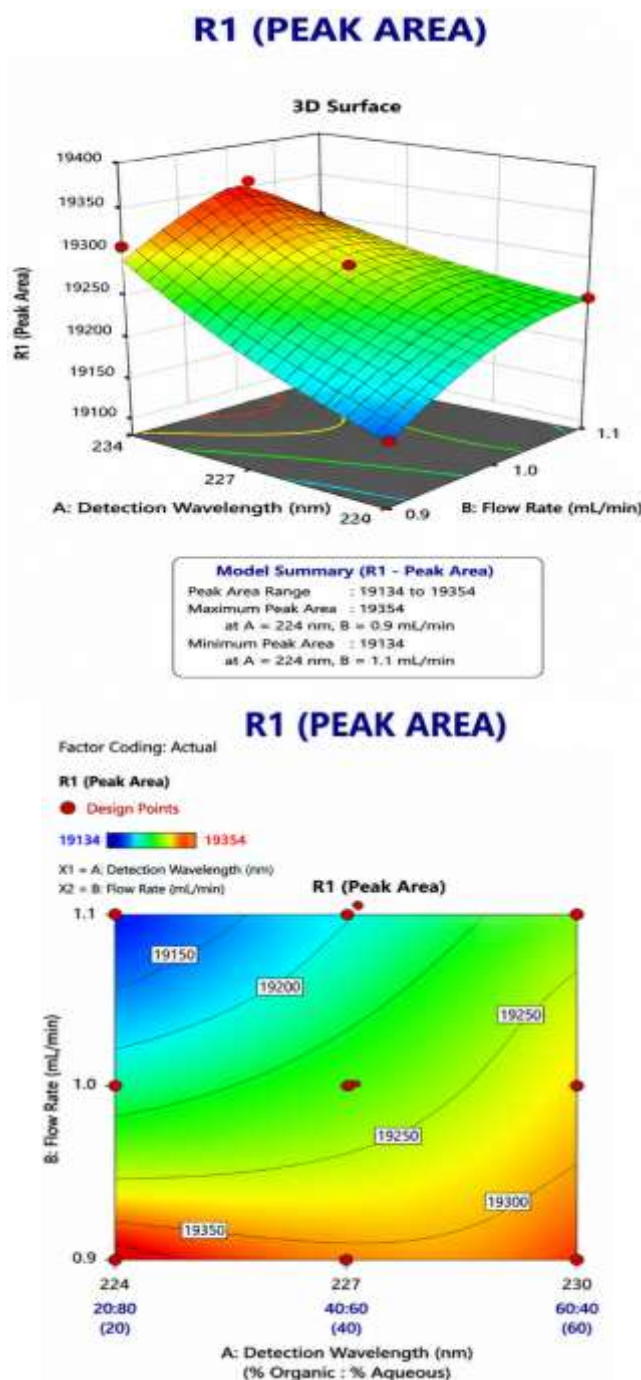


Figure 3. Peak-area response across the nine supplied QbD experiments.

4.4 Effect on theoretical plates

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Theoretical plate counts ranged from 7,388 to 7,632, indicating relatively consistent column efficiency throughout the investigated experimental region. B1 gave the highest plate count, while B8 gave the lowest. The reported model was significant ($F=12.50$, $p=0.0095$), with wavelength and flow rate identified as significant factors.

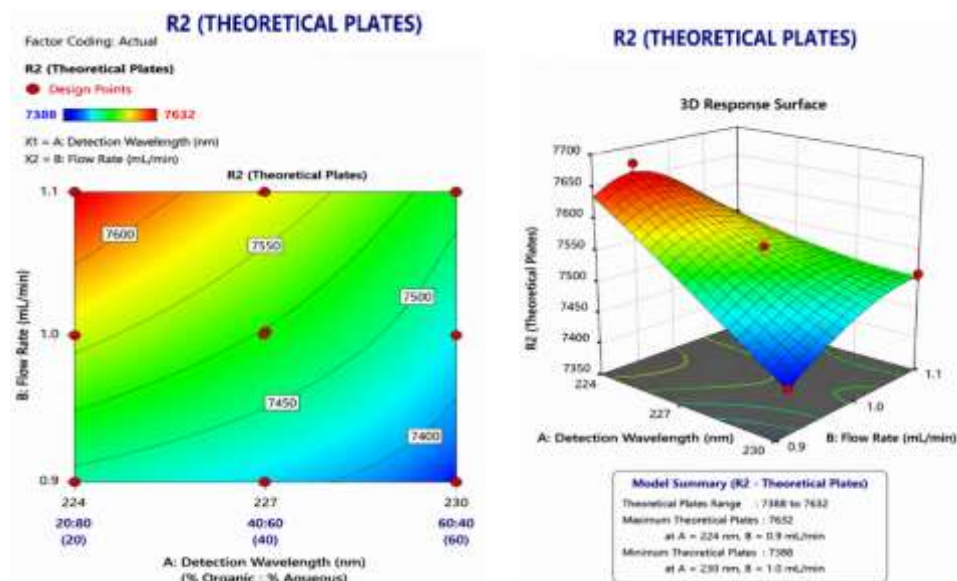


Figure 4. Theoretical-plate response across the nine supplied QbD experiments.

4.5 Effect on asymmetry

Asymmetry values remained between 1.24 and 1.31, indicating satisfactory peak symmetry within the reported system-suitability range. The lowest value was observed for B3 (224 nm, 1.1 mL/min), while the highest values of 1.31 occurred in B1, B6 and B9. The reported asymmetry model F-value was 15.83; wavelength, flow rate and their interaction were reported to contribute significantly.

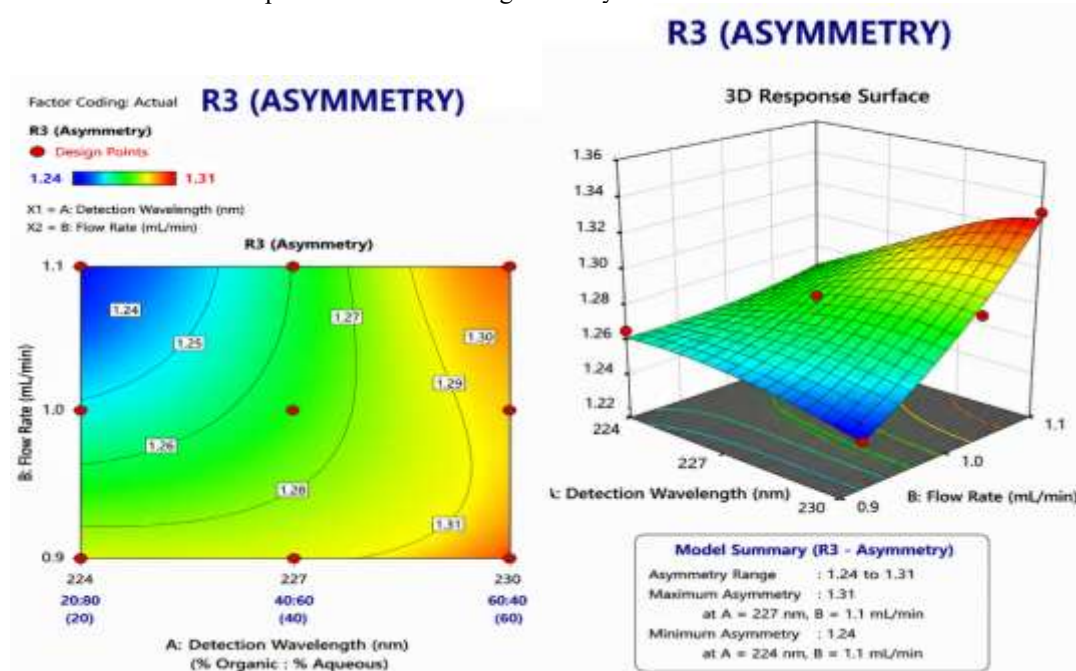


Figure 5. Peak-symmetry response across the nine supplied QbD experiments.

4.6 Effect on retention time

Retention time ranged from 3.13 to 3.21 min in the supplied nine-run dataset. The shortest observed retention time

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was 3.13 min at 227 nm and 1.1 mL/min (B6), while the longest was 3.21 min at 230 nm and 0.9 mL/min (B7). The reported retention-time model was highly significant ($F=65.0$, $p<0.0001$), with wavelength, flow rate and their interaction identified as significant contributors.

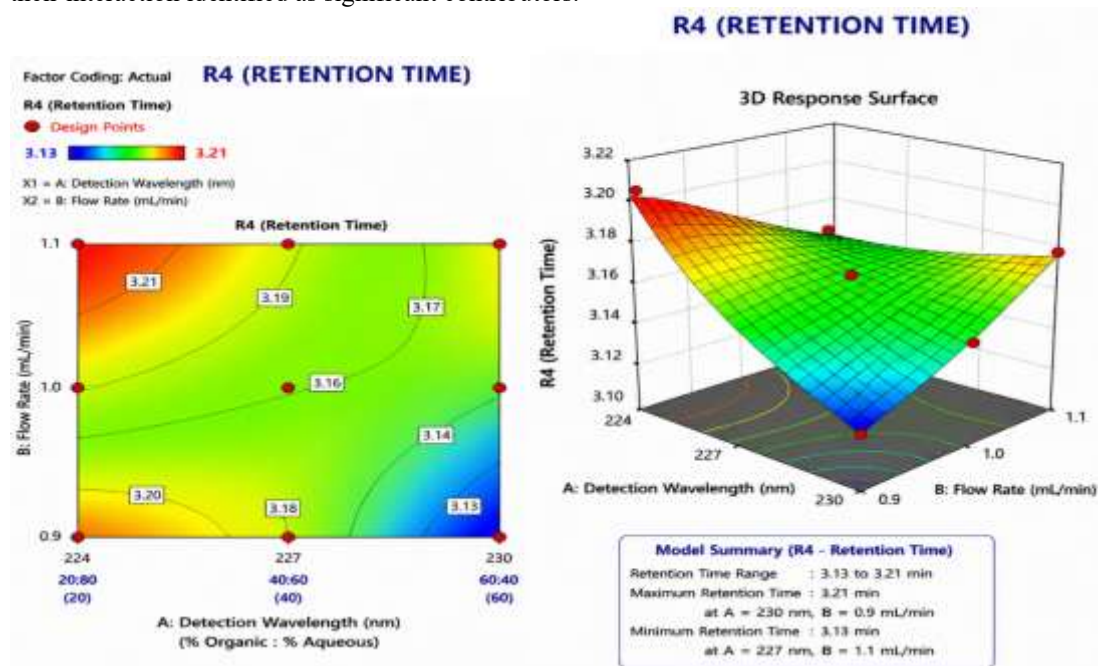


Figure 6. Retention-time response across the nine supplied QbD experiments.

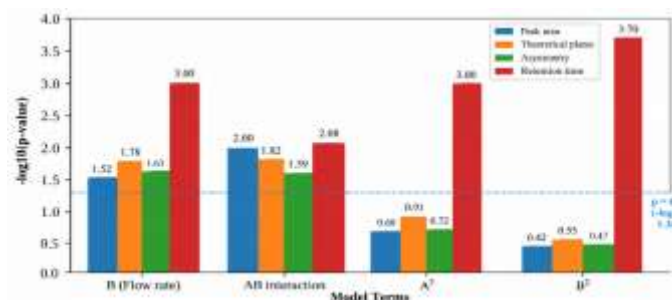


Figure 7. Relative statistical significance of the principal model terms based on reported p-values ($-\log_{10} p$)

4.7 ANOVA

Table 3. ANOVA summary for the four chromatographic responses.

Response	Model F-value	Model p-value	Significant terms reported
Peak area (R1)	6.86	0.0319	Wavelength $\times$ flow-rate interaction ($p=0.0063$)
Theoretical plates (R2)	12.50	0.0095	Wavelength and flow rate
Asymmetry (R3)	15.83	0.0500	Wavelength, flow rate and interaction
Retention time (R4)	65.00	<0.0001	Wavelength, flow rate and interaction

The ANOVA results demonstrate that both investigated method variables influenced at least some chromatographic responses and that the interaction term was important for peak area, asymmetry and retention behavior. This supports the use of interaction-aware DoE rather than relying solely on one-factor-at-a-time experimentation.^{6,7}

4.8 Response-surface analysis

The supplied documents refer to response-surface/DoE evaluation, but the complete numerical response-surface equations and model statistics were not supplied. Therefore, no numerical polynomial equations or interpolated response surfaces have been fabricated in this manuscript. For a final QbD submission, the original Design-Expert output should be inserted, including model equations, coefficient estimates, R^2 , adjusted R^2 , predicted R^2 , lack-of-fit and desirability.

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4.9 Optimization

The published source procedure selected 228 nm and 1.0 mL/min with the phosphate buffer–acetonitrile mobile phase described above.¹⁰ The progress-report wavelength levels, however, were 224, 227 and 230 nm; consequently, 228 nm lies outside that specific nine-run matrix. The manuscript therefore treats 228 nm as the published final operating condition rather than claiming that the supplied nine-run table independently proves 228 nm as its optimum.

4.10 Design space

A formally bounded Method Operable Design Region was not available in the supplied documents. The experimentally studied domain was 224–230 nm and 0.9–1.1 mL/min in the progress report. This should be described as the investigated experimental region, not as a validated numerical design space.

4.11 Verification of optimized conditions

Independent confirmatory verification runs and predicted-versus-observed response statistics were not supplied. Confirmatory injections using independent standard and sample preparations should therefore be performed before submission if the manuscript is intended to make a complete QbD optimization claim.

5. METHOD VALIDATION

5.1 System suitability

The source method specified resolution not less than 2.0, tailing factor not more than 1.5, theoretical plate count not less than 3000 and peak-area %RSD not more than 2.0% for replicate standard injections.¹⁰

Table 4. Reported system-suitability criteria for empagliflozin.

Parameter	Acceptance criterion
Resolution	NLT 2.0
Tailing factor	NMT 1.5
Theoretical plates	NLT 3000
Peak-area %RSD	NMT 2.0%

Note: The supplied manuscript extract provides the acceptance criteria but not a complete replicate-by-replicate system-suitability result table. The original chromatographic report should be inserted before submission.

5.2 Specificity

Specificity was assessed by comparison of the empagliflozin peak retention time in standard and test solutions and by peak-purity assessment. The supplied article reports comparable retention times and a peak-purity acceptance criterion of not less than 97%.¹⁰

5.3 Linearity

Linearity was evaluated over 20–160 µg/mL. The source article reports the calibration relationship as $y = 189532x$ with $R^2 = 0.9899$; the intercept was not reported and has not been reconstructed.¹⁰

5.4 Accuracy

Accuracy was evaluated at 50%, 100% and 150% levels. The reported recoveries were 99.72%, 98.06% and 99.25%, respectively, with an overall reported recovery of 99.80%.¹⁰

Table 5. Accuracy/recovery results reported in the supplied source study.

Recovery level	Drug added (µg/mL)	Mean amount found (µg/mL)	% Recovery
50%	50	148.32 ± 0.66	99.72
100%	100	196.21 ± 0.45	98.06
150%	150	297.77 ± 0.11	99.25

Data-verification note: the “mean amount found” values shown above are reproduced from the supplied source table, but they do not appear internally consistent with the corresponding added concentrations and recovery percentages. They must be checked against the original calculation sheet before submission.

5.5 Precision

The reported intraday and interday precision were 1.14% and 1.12% RSD, respectively. Assay precision based on six test solutions was reported as 1.4% RSD, within the stated NMT 2.0% criterion.¹⁰

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Table 6. Precision results reported in the supplied source study.

Parameter	Result	Acceptance criterion
Intraday precision	1.14% RSD	NMT 2.0%
Interday precision	1.12% RSD	NMT 2.0%
Assay precision	1.4% RSD	NMT 2.0%

5.6 LOD

LOD was calculated using the reported relationship $LOD = 3.3\sigma/S$, where σ represents response standard deviation and S is the calibration-curve slope. The reported LOD was 0.312 $\mu\text{g/mL}$.¹⁰

5.7 LOQ

LOQ was calculated using $LOQ = 10\sigma/S$. The reported LOQ was 0.662 $\mu\text{g/mL}$.¹⁰

5.8 Robustness

The nine-run QbD experiment demonstrates consistency of chromatographic responses over the investigated wavelength and flow-rate combinations and therefore supports a robustness-oriented development strategy. However, variation across DoE factor levels should not be confused with a dedicated formal robustness study involving deliberate small changes around the final method settings. If required by the target journal or regulatory context, a dedicated robustness study should be performed and reported.

6. SOLUTION STABILITY

6.1 Test solution stability

Test-solution stability was evaluated at 0 and 6 h. Retention time changed from 3.19 to 3.21 min, peak area from 19,286 to 19,315, theoretical plates from 7,592 to 7,613 and asymmetry from 1.28 to 1.30. The reported absolute difference was 0.86%, within the NMT 2.0% criterion.¹⁰

6.2 Standard solution stability

The difference in standard-solution recovery after 6 h was reported as 1.21%, also within the NMT 2.0% criterion.¹⁰

Table 7. Stability of standard and test solutions.

Solution	Parameter	0 h	6 h	Acceptance
Test	Retention time (min)	3.19	3.21	—
Test	Peak area	19,286	19,315	—
Test	Theoretical plates	7,592	7,613	—
Test	Asymmetry	1.28	1.30	<2.0
Test	Absolute difference (%)	0.0	0.86	NMT 2.0%
Standard	Recovery difference (%)	0.0	1.21	NMT 2.0%

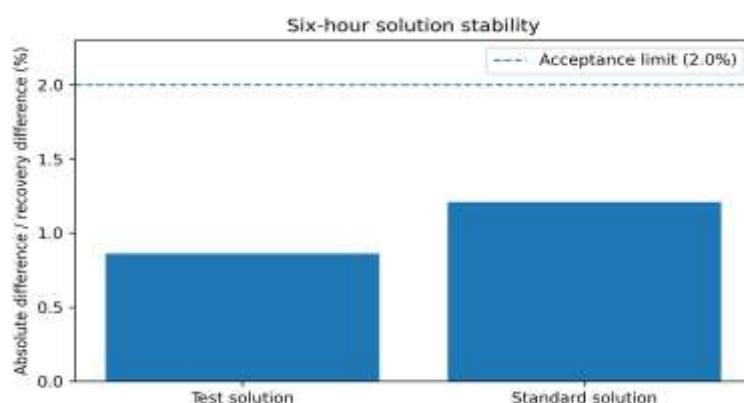


Figure 7. Six-hour solution-stability differences reported for test and standard solutions.

7. ASSAY OF PHARMACEUTICAL FORMULATION

The supplied source article indicates applicability to pharmaceutical dosage forms and reports assay precision of 1.4% RSD.¹⁰ However, the supplied manuscript extract does not provide the complete marketed-product sample-

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preparation sequence, label claim, tablet brand, individual assay values or mean assay result. These formulation-specific details should be inserted from the original laboratory record before the manuscript is presented as a complete pharmaceutical-product application study.

8. CONCLUSION

QbD-oriented RP-HPLC development strategy was used to organize the available empirical and validation information for quantitative determination of empagliflozin. The nine-run dataset demonstrated relatively narrow variation in peak area, theoretical plates, asymmetry and retention time over the investigated wavelength and flow-rate region. Reported ANOVA results indicated statistically significant effects for the major response models and highlighted the importance of factor interactions. The published final procedure used a Discovery C18 column, 0.05 M potassium dihydrogen phosphate buffer at pH 3.0:acetonitrile (40:60, v/v), 1.0 mL/min, 228 nm, 40°C and 20 µL injection volume, with a reported 2.31-min retention time. Validation results showed acceptable linearity, precision, recovery, sensitivity, specificity and six-hour solution stability. Before submission, the wavelength/factor-level discrepancy, the accuracy-table calculations, complete system-suitability data, independent optimization verification and formulation sample-preparation details should be reconciled with the original experimental records.

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