



Quality by Design-assisted development and validation of a robust RP-HPLC method for quantitative determination of ivabradine hydrochloride: (HCN) channel blocker

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ABSTRACT:

Ivabradine hydrochloride is a cardiovascular drug requiring reliable quantitative estimation for pharmaceutical quality control. Quality by Design (QbD) strategy was applied to develop and optimize a reversed-phase high-performance liquid chromatographic (RP-HPLC) method for ivabradine hydrochloride. Preliminary chromatographic trials supported selection of a C18 stationary phase, potassium dihydrogen phosphate–acetonitrile mobile phase, 288 nm detection, and a short chromatographic run time. Mobile-phase ratio and flow rate were investigated as experimental factors at three levels using nine experimental runs. Peak area, theoretical plate count, asymmetry and retention time were selected as analytical responses. Retention time ranged from 3.05 to 3.10 min, peak area from 2420 to 2675, theoretical plates from 7544 to 7716, and asymmetry from 1.31 to 1.35. ANOVA demonstrated significant effects of flow rate and the mobile-phase ratio $\times$ flow-rate interaction for peak area, theoretical plates and asymmetry, while retention time was significantly influenced by flow rate, the interaction term and quadratic effects. The selected chromatographic condition comprised a 35:65 (v/v) ratio of 0.1 M potassium dihydrogen phosphate and acetonitrile, a flow rate of 1.0 mL/min, column temperature of 40°C, 20 μ L injection volume and detection at 288 nm. The method showed satisfactory system suitability, linear response over 10–100 μ g/mL, recoveries of 99.3–100.9%, and assay precision of 100.4% with 1.0% RSD. Standard and test solutions remained stable for 6 h, with differences of 1.30% and 0.58%, respectively. The findings demonstrate that QbD-assisted optimization can provide a rapid and reproducible RP-HPLC procedure for routine estimation of ivabradine hydrochloride. LOD, LOQ, formal robustness experiments, formulation sample-preparation details, and independent optimization verification were not reported in the supplied source documents and should be completed before final submission.

1. INTRODUCTION:

Ivabradine hydrochloride is a cardiovascular drug that selectively inhibits the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel-mediated pacemaker current in the sinoatrial node. Its action decreases the rate

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of spontaneous diastolic depolarization and thereby reduces heart rate without directly reducing myocardial contractility.¹ Accurate and reproducible estimation of ivabradine hydrochloride is important for pharmaceutical quality control. RP-HPLC is particularly suitable for quantitative analysis because chromatographic conditions can be optimized to provide rapid analysis, adequate peak shape and reproducible response.^{1,2}

A previously reported RP-HPLC procedure for ivabradine hydrochloride employed a C18 column, 0.1 M potassium dihydrogen phosphate and acetonitrile in a 35:65 ratio, a flow rate of 1.0 mL/min, detection at 288 nm and a 3-min run time. The reported method showed satisfactory system suitability, linearity, accuracy, precision and specificity.¹ Conventional chromatographic development frequently relies on sequential experimentation and may not adequately characterize interactions among method variables. A QbD strategy integrates predefined analytical objectives, factor identification, experimental design and statistical evaluation to improve method understanding and control.^{2,3}

Design of Experiments (DoE) permits simultaneous evaluation of multiple factors and their interactions and can be used to develop response models and identify suitable operating conditions. Response-surface methodology is therefore useful for systematic optimization of chromatographic variables.^{3,4}

Although the available ivabradine hydrochloride study established a satisfactory RP-HPLC method, the supplied Sixth Report provides a more explicit factor–response investigation involving mobile-phase ratio and flow rate and four chromatographic responses. The present manuscript organizes these findings into an QbD-oriented analytical development framework. A method-development strategy that considers peak response, column efficiency, peak symmetry and retention time simultaneously is expected to provide a more comprehensive assessment of chromatographic performance than optimization based on a single response.

The aim was to develop and optimize a rapid RP-HPLC method for ivabradine hydrochloride using a QbD-based experimental strategy. The objectives were to evaluate mobile-phase ratio and flow rate, characterize their effects on critical chromatographic responses, identify suitable operating conditions, and assess the available validation and solution-stability data.

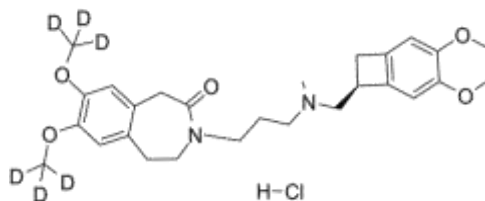


Figure 1: Structure of Ivabradine hydrochloride

2. MATERIALS AND METHODS

2.1 Materials

Ivabradine hydrochloride was obtained as a gift sample from Sun Pharma, Vadodara, Gujarat, India. HPLC-grade chemicals were obtained from Merck. Potassium dihydrogen phosphate and acetonitrile were used for preparation of the mobile phase and analytical solutions.¹

2.2 Instrumentation

Chromatographic analysis was performed using an Agilent 1260 Infinity HPLC system equipped with an autosampler (AS-4050), PDA detector and degasser. OpenLab/EZChrom software was used for data acquisition and processing.¹

2.3 Chromatographic conditions

A C18 stationary phase was selected after preliminary chromatographic trials. The optimized mobile phase consisted of 0.1 M potassium dihydrogen phosphate and acetonitrile in a 35:65 (v/v) ratio. The flow rate was 1.0 mL/min, column temperature 40°C, injection volume 20 µL and total run time approximately 3 min. The mobile-phase components were filtered through a 0.45-µm membrane and sonicated for 15 min before use.¹

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2.4 Detection wavelength

Detection was performed at 288 nm using the PDA detector.¹

2.5 Column selection

A C18 column was selected during preliminary chromatographic investigations on the basis of chromatographic performance and system-suitability characteristics.¹

2.6 Mobile-phase selection

Different proportions of methanol, water, acetonitrile and buffer systems at different pH conditions were investigated during preliminary trials. The 35:65 (v/v) potassium dihydrogen phosphate–acetonitrile composition produced satisfactory peak characteristics at 1.0 mL/min.¹

2.7 Standard preparation

A 1000 µg/mL stock solution was prepared by accurately weighing 10 mg of ivabradine hydrochloride into a 10-mL volumetric flask and making up the volume with the selected mobile phase. A 100 µg/mL working solution was prepared by transferring 1 mL of stock solution to a 10-mL volumetric flask and diluting to volume with the mobile phase, followed by sonication for 10 min.¹

2.8 Sample preparation

The supplied source article states that the method was intended for bulk drug and tablet dosage forms; however, a complete tablet sample-preparation procedure and formulation-specific weighing/dilution details were not provided in the supplied material. These details must therefore be inserted from the original laboratory record before submission.

2.9 Statistical analysis

The nine experimental runs were evaluated using the reported Design-Expert factorial design. Analysis of variance (ANOVA) was used to identify significant model terms at $p < 0.05$. The four responses were peak area, theoretical plates, asymmetry and retention time.^{3,4}

3. QbD-BASED METHOD DEVELOPMENT

3.1 Analytical Target Profile

The analytical target was a rapid and reproducible RP-HPLC procedure capable of providing an adequately intense and symmetric ivabradine hydrochloride peak, satisfactory column efficiency and short retention time for routine quantitative analysis. The experimentally monitored responses were peak area, theoretical plates, asymmetry and retention time.

3.2 Risk assessment

The Sixth Report did not provide a formal numerical risk-assessment matrix or risk-priority-number calculation. Accordingly, no unsupported risk scores have been introduced in this manuscript. The two variables selected experimentally—mobile-phase ratio and flow rate—are treated as study factors whose effects were evaluated statistically.

3.3 Critical Method Parameters

Mobile-phase ratio (A) and flow rate (B) were selected as the independent chromatographic variables. Mobile-phase ratio was studied at 25:75, 35:65 and 45:55, while flow rate was studied at 0.8, 1.0 and 1.2 mL/min.

3.4 Critical Analytical Responses

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

3.5 Design of Experiments

A two-factor, three-level factorial design was employed to investigate the factor–response relationships. Nine experimental combinations (M1–M9) were evaluated.

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3.6 Experimental design

Table 1. Experimental design and observed chromatographic responses.

Run	Mobile phase (A)	Flow rate (B), mL/min	Peak area	Theoretical plates	Asymmetry	RT, min
M1	25:75	0.8	2521	7544	1.31	3.08
M2	25:75	1.0	2530	7605	1.32	3.07
M3	25:75	1.2	2517	7716	1.35	3.08
M4	35:65	0.8	2543	7640	1.31	3.08
M5	35:65	1.0	2546	7611	1.33	3.05
M6	35:65	1.2	2523	7688	1.35	3.10
M7	45:55	0.8	2675	7646	1.32	3.07
M8	45:55	1.0	2542	7617	1.31	3.07
M9	45:55	1.2	2420	7634	1.31	3.09

4. RESULTS AND DISCUSSION

4.1 Preliminary method development

Preliminary trials evaluated different mobile-phase compositions and flow rates. The final chromatographic system used a C18 column with 0.1 M potassium dihydrogen phosphate–acetonitrile (35:65, v/v), 1.0 mL/min flow, 40°C column temperature and 288 nm detection. The resulting analysis was completed within approximately 3 min, providing a suitable basis for systematic optimization.¹

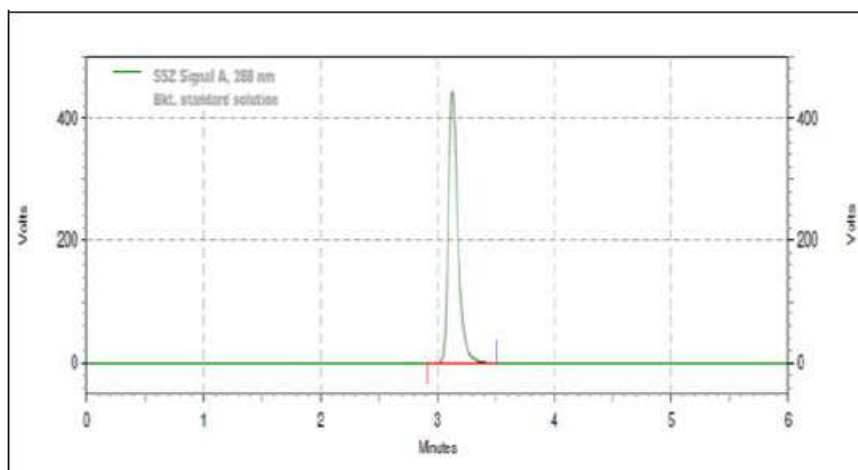


Figure 1. Observed response profile across the nine experimental runs. The plot summarizes peak-area and retention-time behavior

4.2 Experimental design results

The nine experimental runs produced relatively narrow ranges for the four responses. Retention time ranged from 3.05 to 3.10 min, peak area from 2420 to 2675, theoretical plates from 7544 to 7716 and asymmetry from 1.31 to 1.35. These results indicate that the chromatographic system maintained acceptable performance throughout the investigated factor space.

4.3 Effect on peak area

Peak area ranged from 2420 to 2675. The highest response was observed for M7 (45:55, 0.8 mL/min), whereas the lowest response was observed for M9 (45:55, 1.2 mL/min). The report noted a slight reduction in peak response at the higher flow rate and relatively consistent response for the 35:65 mobile-phase ratio. These observations were supported by ANOVA, in which flow rate and the A×B interaction were significant.

4.4 Effect on theoretical plates

Theoretical plate count ranged from 7544 to 7716, indicating good column efficiency for all experimental conditions. M3 (25:75, 1.2 mL/min) gave the highest plate count, whereas M1 (25:75, 0.8 mL/min) gave the lowest. The statistical model was significant ($F=4.56$, $p=0.0348$), with flow rate ($p=0.0317$) and the A×B interaction ($p=0.0163$) identified as significant terms.

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4.5 Effect on asymmetry

Asymmetry values remained between 1.31 and 1.35, well below the reported acceptance criterion of 2.0. A small increase was observed at 1.2 mL/min. The ANOVA model was significant ($F=6.51$, $p=0.0148$); flow rate ($p=0.0172$) and the $A \times B$ interaction ($p=0.0263$) were significant, whereas mobile-phase ratio alone was not significant ($p=0.1282$).

4.6 Effect on retention time

Retention time showed minimal variation, from 3.05 to 3.10 min, with an overall mean of approximately 3.07 min. M5 gave the shortest retention time (3.05 min) and M6 the longest (3.10 min). The retention-time model was highly significant ($F=100.21$, $p=0.0012$). Flow rate, the $A \times B$ interaction and both quadratic terms (A^2 and B^2) were significant, demonstrating a nonlinear factor–response relationship for retention time.

4.7 ANOVA

Table 2. ANOVA summary for the four chromatographic responses.

Response	Model F	Model p	Significant terms ($p < 0.05$)
Peak area (R1)	5.26	0.0257	B (0.0310), AB (0.0101)
Theoretical plates (R2)	4.56	0.0348	B (0.0317), AB (0.0163)
Asymmetry (R3)	6.51	0.0148	B (0.0172), AB (0.0263)
Retention time (R4)	100.21	0.0012	B (0.0010), AB (0.0086), A^2 (0.0010), B^2 (0.0002)

The ANOVA results demonstrate that flow rate was the most consistently influential factor because it was significant for all four responses. The interaction between mobile-phase ratio and flow rate was also significant for all responses, indicating that the effect of one factor depended on the level of the other. Retention time additionally showed significant quadratic effects, supporting the use of response-surface modeling for this response.^{3,4}

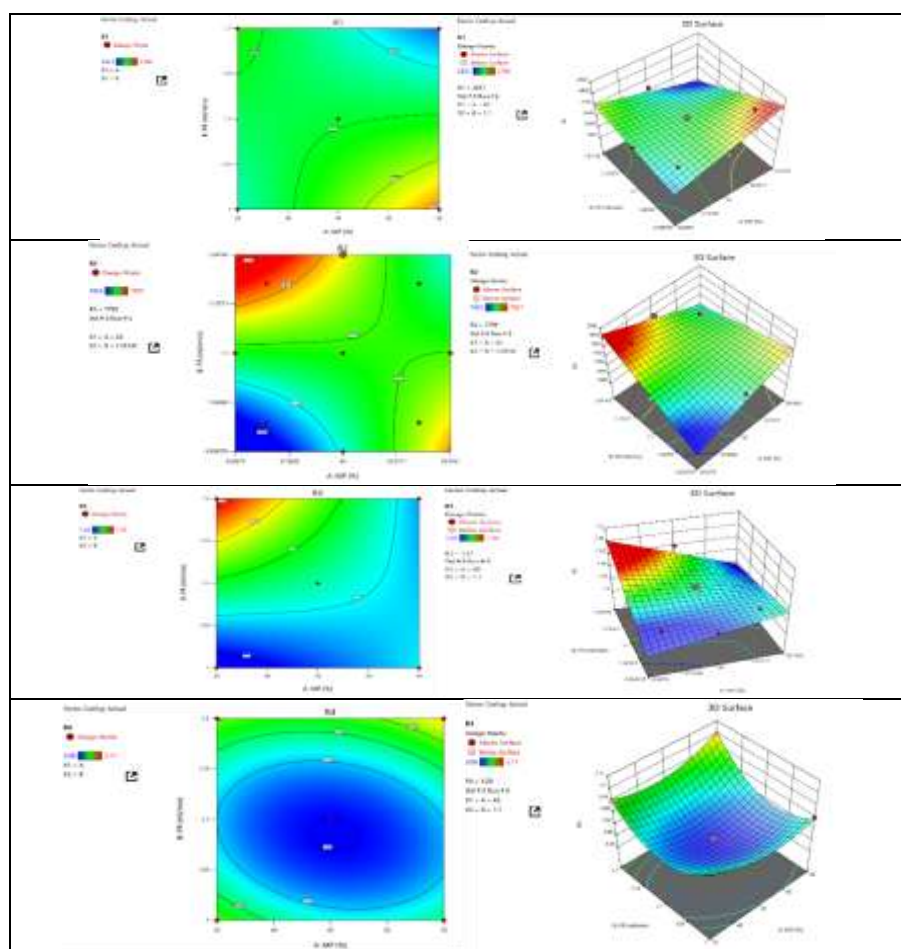


Figure 2. Observed response profiles for peak area, theoretical plates, asymmetry and retention time at the three mobile-phase ratios

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and three flow rates.

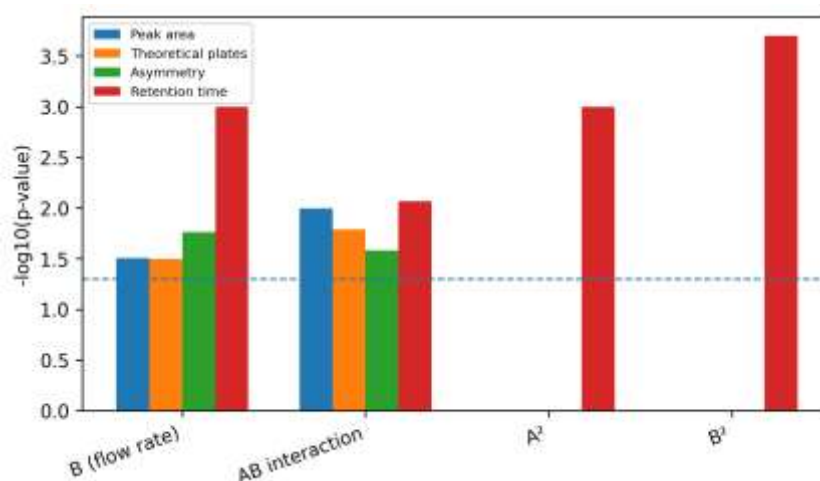


Figure 3. Relative statistical significance of the principal model terms based on reported p-values ($-\log_{10} p$).

4.8 Response-surface analysis

The factor–response patterns indicate that the chromatographic responses were influenced by the combined settings of mobile-phase ratio and flow rate rather than by either variable alone. In particular, the significance of the A×B interaction across all four responses demonstrates the value of simultaneous factor evaluation. The source report refers to response-surface analysis; however, the numerical response-surface equations and predicted response values were not available in the supplied text and are therefore not reproduced here.

4.9 Optimization

Considering the combined chromatographic performance, the selected operating condition was a 35:65 (v/v) ratio of 0.1 M potassium dihydrogen phosphate and acetonitrile with a flow rate of 1.0 mL/min. This condition corresponds to M5 and provided a retention time of 3.05 min, peak area of 2546, theoretical plates of 7611 and asymmetry of 1.33. The condition also agrees with the optimized conditions reported in the published ivabradine hydrochloride study.¹

4.10 Design space

A formal numerical design-space boundary was not reported in the supplied Sixth Report. The experimental region investigated was 25:75–45:55 mobile-phase ratio and 0.8–1.2 mL/min flow rate. Therefore, this region should be described as the studied experimental domain rather than as a formally established design space.

4.11 Verification of optimized conditions

Independent confirmatory verification runs and predicted-versus-observed response statistics were not reported in the supplied documents. The selected condition should therefore be experimentally verified with independent preparations before the manuscript is submitted as a complete QbD study.

5. METHOD VALIDATION

5.1 System suitability

The reported system-suitability results were satisfactory. Theoretical plates were 8762 (>1000), tailing factor was 0.681 (<2), area %RSD was 0.158% (<2%) and retention-time %RSD was 0.164% (<1%). These results demonstrate adequate column efficiency, peak symmetry and injection reproducibility.¹

Table 3. System suitability results for ivabradine hydrochloride.

Parameter	Acceptance criterion	Observed value
Theoretical plates	>1000	8762
Tailing factor	<2	0.681
Area %RSD	<2%	0.158%
RT %RSD	<1%	0.164%

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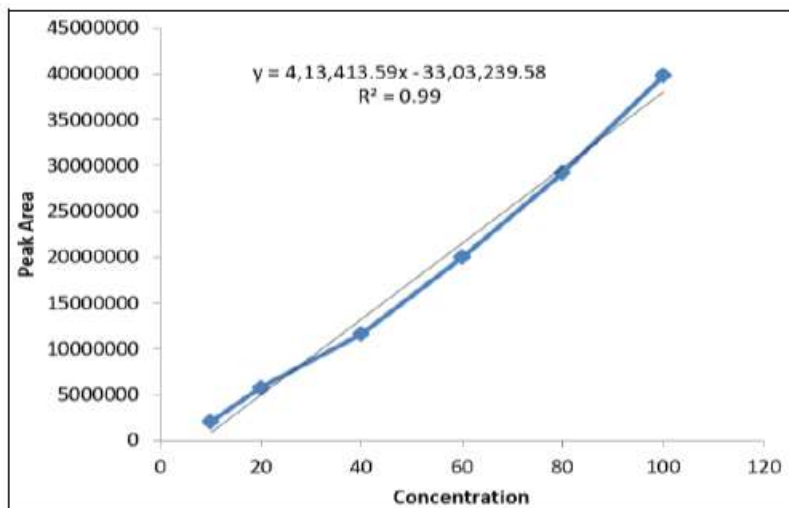
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5.2 Specificity

Specificity was evaluated by comparison of the retention time of the drug peak in standard and test solutions and by peak-purity assessment. The reported acceptance criterion for peak purity was not less than 96.0%, and the test peak retention time was reported to be comparable with that of the standard.¹

5.3 Linearity

Linearity was investigated over the concentration range of 10–100 µg/mL. The reported calibration relationship was $y=413414x+3,303,239.58$ with a reported regression value of 1.00. The response therefore showed a strong concentration–peak-area relationship over the studied range.¹



5.4 Accuracy

Accuracy was evaluated at 50%, 100% and 150% levels. Recoveries were 99.3%, 100.9% and 99.5%, respectively, indicating close agreement between added and measured concentrations.¹

Table 4. Accuracy results for ivabradine hydrochloride.

Assay level (%)	Recovery (%)
50	99.3
100	100.9
150	99.5

5.5 Precision

Six test solutions gave a mean assay value of 100.4%, with 1.0% RSD. Both results complied with the reported acceptance criteria of 98–102% for assay and not more than 2.0% RSD.¹

Table 5. Precision results for ivabradine hydrochloride.

Parameter	Result	Acceptance criterion
Mean assay	100.4%	98–102%
%RSD	1.0%	NMT 2.0%

5.6 LOD

LOD data were not reported in the supplied Ivabradine HCl article or Sixth Report. A validated LOD experiment should be added before final submission.

5.7 LOQ

LOQ data were not reported in the supplied Ivabradine HCl article or Sixth Report. A validated LOQ experiment should be added before final submission.

5.8 Robustness

The Sixth Report describes consistent retention time across the nine DoE conditions, but this is not equivalent to a formal robustness experiment. A dedicated robustness study involving deliberate small changes in critical

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chromatographic conditions should be performed and reported before claiming formal robustness.

6. SOLUTION STABILITY

6.1 Test solution stability

Test-solution stability was assessed at 0 and 6 h. Retention time changed from 3.09 to 3.15 min, peak area from 26,630,264 to 26,788,055, theoretical plates from 7763 to 7643 and asymmetry from 1.41 to 1.35. The absolute difference between the initial and 6-h results was 0.58%, within the NMT 2.0% criterion. The report therefore considered the test solution stable for at least 6 h under the studied conditions.⁵

6.2 Standard solution stability

The difference in standard-solution recovery after 6 h was 1.30%, which was within the NMT 2.0% acceptance criterion. The standard solution was consequently considered stable for 6 h under the investigated conditions.⁵

Table 6. Stability of standard and test solutions.

Solution	Parameter	0 h	6 h	Acceptance
Test	Retention time (min)	3.09	3.15	—
Test	Peak area	26,630,264	26,788,055	—
Test	Theoretical plates	7763	7643	—
Test	Asymmetry	1.41	1.35	<2.0
Test	Absolute difference (%)	0.0	0.58	NMT 2.0%
Standard	Recovery difference (%)	0.0	1.30	NMT 2.0%

7. ASSAY OF PHARMACEUTICAL FORMULATION

The source article states that the method was intended for assay of ivabradine hydrochloride in bulk and tablet dosage forms. Six test solutions produced an average assay of 100.4% with 1.0% RSD, satisfying the reported assay range of 98–102% and precision criterion of NMT 2.0%. However, the supplied material does not provide the tablet brand, label claim, tablet weighing, extraction volume or dilution sequence. These formulation-specific details should be inserted from the original experimental record before submission.¹

8. COMPARISON WITH REPORTED METHODS

The principal source study reported a C18 RP-HPLC method using 0.1 M potassium dihydrogen phosphate and acetonitrile (35:65), 1.0 mL/min flow, 288 nm detection and approximately 3 min run time. The present manuscript uses the same experimental dataset but places greater emphasis on the four-response DoE analysis and the factor–response relationships documented in the Sixth Report. Because no independent alternative ivabradine datasets were supplied, a numerical inter-method comparison cannot be performed without adding additional published methods.¹

Table 7. Comparison with the supplied published ivabradine hydrochloride study.

Parameter	Present QbD dataset	Published ivabradine study
Stationary phase	C18	C18
Mobile phase	0.1 M KH ₂ PO ₄ :ACN (35:65)	0.1 M KH ₂ PO ₄ :ACN (35:65)
Flow rate	1.0 mL/min	1.0 mL/min
Detection	288 nm	288 nm
Run time	~3 min	3 min
Linearity	10–100 µg/mL	10–100 µg/mL
Recovery	99.3–100.9%	99.3–100.9%

9. CONCLUSION:

A QbD-oriented RP-HPLC development strategy was applied to evaluate the effects of mobile-phase ratio and flow rate on the chromatographic performance of ivabradine hydrochloride. Nine experimental runs demonstrated narrow variation in retention time, satisfactory peak symmetry and high theoretical plate counts. ANOVA identified flow rate and the mobile-phase ratio × flow-rate interaction as significant contributors to peak area, theoretical plates and asymmetry, while retention time was additionally affected by quadratic terms. The selected condition of 0.1 M potassium dihydrogen phosphate–acetonitrile (35:65, v/v), 1.0 mL/min, 40°C and 288 nm provided rapid analysis and satisfactory reported validation performance. Standard and test solutions were stable for 6 h. However, formal LOD, LOQ, robustness, design-space verification and complete formulation sample-preparation data were not available in the supplied documents and should be completed before submission.

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