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Original Research Article

QbD approach to HPLC method development and validation of the simultaneous estimation of sulbactam and durlobactam in pharmaceutical dosage form

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Abstract

Background: Sulbactam (SLM) and durlobactam (DOM) are β-lactamase inhibitors co-formulated for the treatment of multidrug-resistant *Acinetobacter baumannii* infections. A robust, reproducible, and regulatory-compliant analytical method is required for their routine quality control.

Objective: To develop and validate a stability-compliant RP-HPLC method for the simultaneous estimation of SLM and DOM in pharmaceutical dosage forms using the Analytical Quality by Design (AQbD) framework.

Methods: Critical method parameters were identified using Ishikawa diagram and FMEA. A Box–Behnken design (BBD) with 17 randomized runs was applied to study the effects of flow rate, pH, and organic phase proportion on resolution (Y_1) and tailing factor (Y_2). The method was optimized using response surface modelling, and the method operable design region (MODR) was established. The optimized conditions employed a Hypersil Gold C18 column (150 × 3.0 mm, 3 µm), methanol:0.1% formic acid (65:35, v/v; pH 3.5) as mobile phase, flow rate 1.0 mL·min^{-1}, and detection at 220 nm. Validation was performed in line with ICH Q2(R1).

Results: Resolution was predominantly influenced by organic proportion and flow rate, while tailing was affected by curvature near MODR boundaries. The selected operating point (65% methanol, pH 3.5, 1.0 mL·min^{-1}) yielded sharp, symmetric peaks (Rt: 2.16 min for SLM; 3.44 min for DOM) with resolution ≥ 2.0. Validation confirmed linearity (10–50 µg·mL^{-1}, R^2 = 0.999), precision (%RSD < 2%), accuracy (98–102%), robustness, and sensitivity (LOD: 0.38 µg·mL^{-1} SLM, 0.08 µg·mL^{-1} DOM). System suitability criteria were consistently met.

Conclusion: The developed RP-HPLC method is simple, precise, accurate, and robust, fulfilling AQbD and ICH Q2(R1) requirements. Its reliance on readily available reagents and UV detection makes it highly suitable for routine quality control and regulatory compliance. Future work should expand to forced degradation and peak purity studies to confirm stability-indicating performance.

Keywords: Analytical Quality by Design, RP-HPLC, Sulbactam, Durlobactam, Method Validation, Box–Behnken Design.

Received: 11-08-2025**; Accepted:** 18-09-2025**; Available Online:** 08-10-2025

1. Introduction

Quality by Design (QbD) and Process Analytical Technology (PAT) have transformed analytical development by shifting from empirical trial-and-error to proactive, risk-based control strategies that ensure consistent performance across the method lifecycle.[1-6] Analytical QbD (AQbD) translates these principles into the analytical domain by defining an Analytical Target Profile (ATP), identifying Critical Quality Attributes (CQAs), performing risk assessment, conducting Design of Experiments (DoE), establishing a Control Strategy, and confirming a Method Operable Design Region (MODR) with continuous method monitoring.[7-12]

Sulbactam is a β-lactamase inhibitor, commonly used in combination with β-lactam antibiotics to overcome bacterial resistance. Durlobactam, a novel diazabicyclooctane (DBO) β-lactamase inhibitor, shows potent activity against multidrug-resistant Acinetobacter baumannii. The fixed-dose combination of Sulbactam and Durlobactam represents a promising therapeutic strategy in combating carbapenem-resistant infections.[12-14]

Their simultaneous quantification in dosage forms is critical for assay and content uniformity. Prior art reports UPLC/HPLC methods for each or both analytes, yet few explicitly embed AQbD with response-surface optimization

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https://doi.org/10.18231/j.pharmacist.89611.1759917827

and complete ICH validation for a compact 3 µm C18 format enabling sub-4 min Rt for each component.[15-17]

2. Aim and Objective

Develop and validate an AQbD-based RP-HPLC method for simultaneous SLM and DOM estimation in finished products, demonstrating ruggedness and robustness consistent with ICH Q2 (R1).

3. Materials and Methods

3.1. Chemicals and Reagents

Sulbactam (SLM) and Durlobactam (DOM) working standards were obtained from MSN Laboratories, India (purity ≥ 99%). Methanol and acetonitrile (HPLC grade) were procured from Standard Solutions Ltd. Potassium dihydrogen phosphate (analytical grade) was purchased from FINAR, while formic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were supplied by Merck, India. Water used throughout the study was HPLC grade. All chemicals and solvents were used without further purification.

3.2. Instrumentation

Chromatographic separation was carried out on a Waters Alliance 2695 separation module equipped with a 2487 dual-wavelength UV detector, operated using Empower software. Spectral analysis was performed on an Agilent Cary 60 UV–Vis spectrophotometer. The chromatographic separation was achieved using a Hypersil Gold C18 column (150 × 3.0 mm, 3 µm). Buffer pH was adjusted using a calibrated Orion Lab Star pH meter, and weighing was performed on a Mettler Toledo analytical balance.

3.3. Analytical target profile (ATP) and critical quality attributes (CQAs)

The ATP was established to ensure reliable quantification of SLM and DOM in pharmaceutical dosage forms with acceptable separation and accuracy. The criteria included:

1. Resolution ≥ 2.0 between SLM and DOM peaks,
2. Tailing factor ≤ 2.0,
3. Theoretical plates (N) ≥ 2000,
4. Accuracy between 98–102%,
5. Precision (%RSD) ≤ 2.

The CQAs identified were resolution (Y_1), tailing factor (Y_2), retention time, theoretical plates, LOD/LOQ, and method accuracy and precision.

3.4. Risk assessment

A structured Ishikawa diagram was employed to identify potential variables affecting method performance. Failure Mode and Effects Analysis (FMEA) was subsequently applied to prioritize factors. Flow rate, buffer pH, and organic phase ratio were identified as critical method parameters (CMPs), while column temperature and detection wavelength were classified as lower-risk variables, controlled according to ATP specifications.

3.5. Design of experiments (DoE)

A Box–Behnken Design (BBD) with three independent factors at three levels was employed to explore the effects of CMPs using Design Expert software (v22.0.4.0). Seventeen randomized runs were executed. The investigated factors were:

1. A: Flow rate (0.90–1.00 $mL \cdot min^{-1}$),
2. B: Buffer pH (4.00–4.50),
3. C: Organic phase percentage (55–66% v/v).

The selected responses were:

Y_1: Resolution between SLM and DOM,

Y_2: Maximum tailing factor.

Quadratic polynomial models were generated and evaluated using ANOVA. Model adequacy was assessed through residual analysis, predicted versus actual values, and 3D surface response plots. The Method Operable Design Region (MODR) was defined to ensure robust method performance.

3.6. Optimized chromatographic conditions

The optimized separation was achieved using the following chromatographic conditions:

1. Column: Hypersil Gold C18 (150 × 3.0 mm, 3 µm),
2. Mobile phase: Methanol (65%) : aqueous 0.1% formic acid (35%), adjusted to pH 3.5 with NaOH,
3. Flow rate: 1.0 $mL \cdot min^{-1}$,
4. Detection wavelength: 220 nm,
5. Injection volume: 10 µL,
6. Total run time: 10 min.

The diluent consisted of methanol and 0.1% formic acid in a 65:35 (v/v) ratio. All solutions were filtered through a 0.45 µm membrane filter and degassed before use.

3.7. Standard and sample preparation

Standard solution: Approximately 25 mg each of SLM and DOM was weighed accurately and transferred into separate

25 mL volumetric flasks. The drugs were dissolved in diluent, sonicated, and diluted to volume to obtain stock solutions. Working standards were prepared by transferring 0.3 mL of each stock solution into separate 10 mL volumetric flasks and diluting to volume with diluent, yielding ~30 $\mu g \cdot mL^{-1}$ solutions.

Sample solution: Powder equivalent to 25 mg each of SLM and DOM was accurately weighed into a 25 mL volumetric flask, dissolved in diluent, sonicated, and diluted to volume. The resulting solution was further diluted as described for the standards to obtain a test solution equivalent to ~30 $\mu g \cdot mL^{-1}$ of each analyte.

3.8. System suitability

System suitability was evaluated by injecting standard solutions under optimized conditions. Acceptance limits were resolution ≥ 2.0 between SLM and DOM, tailing factor ≤ 2.0, and theoretical plates ≥ 2000. The results (reported in Table 10) confirmed that all parameters complied with criteria, verifying the reliability of the chromatographic system.

3.9. Method validation

The optimized method was validated according to ICH Q2(R1) guidelines:

1. Assay: % label claim determined using an external standard method,
2. Linearity: Assessed in the range of 10–50 $\mu g \cdot mL^{-1}$ at five levels,
3. Precision: Repeatability assessed at working concentration (n = 6),
4. Intermediate Precision: Evaluated on different days and instruments (n = 6),
5. Accuracy: Recovery experiments conducted at 50%, 100%, and 150% levels (n = 3 each),
6. LOD and LOQ: Determined based on signal-to-noise ratios of ~3 and ~10, respectively,
7. Robustness: Studied by deliberate variations in flow rate (0.8 and 1.2 $mL \cdot min^{-1}$) and organic composition (±10%).

4. Results

4.1. DoE model fitting for resolution (Y_1)

The Box–Behnken design (BBD) generated data for resolution between SLM and DOM, which was modeled using regression analysis. The fit summary indicated that a quadratic model best described the response, showing a statistically significant improvement over the two-factor interaction (2FI) model (p = 0.0068).

ANOVA for the quadratic model demonstrated that the model was highly significant, with F = 52.93, p < 0.0001. Significant model terms included organic phase percentage (A; p < 0.0001), flow rate (B; p = 0.0022), interaction terms AB (p = 0.0044) and BC (p = 0.0157), and the quadratic term A^2 (p = 0.0011). Buffer pH (C) showed a marginal contribution (p = 0.0510).

The residual mean square was small (MS = 8.178 × 10^{-4}), and although lack-of-fit was indicated due to the aliased cubic model (expected for BBD with limited pure error), diagnostic plots supported model adequacy. Residuals versus run demonstrated no discernible pattern, and predicted versus actual values aligned closely with the line of identity, confirming reliability.

The 3D response surface plots (**Figure 1**) highlighted that resolution improved at higher organic content (~65%) combined with moderate flow (0.95–1.0 $mL \cdot min^{-1}$), while pH exerted only a minor influence within the studied range.

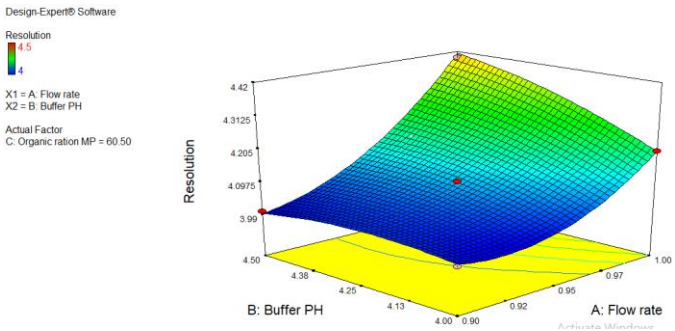

Figure 1: 3D Surface for SLM and DOM

4.2. DoE model fitting for tailing (Y_2)

For tailing factor, the quadratic model was also found appropriate. ANOVA results indicated a significant overall model (F = 9.01, p = 0.0042). Significant contributors included flow rate (B; p = 0.0113), interaction AB (p = 0.0111), and quadratic terms A^2 (p = 0.0035), B^2 (p = 0.0008), and C^2 (p = 0.0140).

Interestingly, the main effects of organic percentage (A) and buffer pH (C) were not individually significant, but curvature terms confirmed sensitivity at extreme conditions. This indicated that peak symmetry was maintained across most of the design space but could deteriorate if the CMPs were operated near their extremes.

Diagnostic plots supported model adequacy, with residuals evenly distributed and predicted versus actual plots showing good correlation. The 3D surface plots (**Figure 2**) illustrated that higher flow rates (>1.0 $mL \cdot min^{-1}$) slightly increased tailing, whereas moderate flow combined with ~65% organic content minimized it.

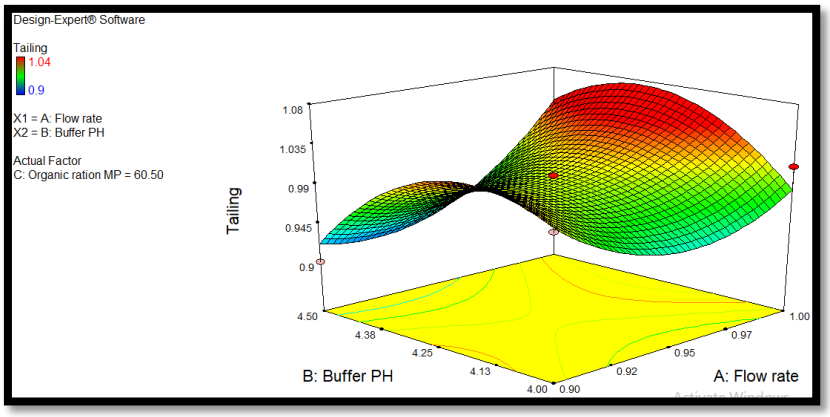

Figure 2: 3D response surface plot showing the influence of flow and organic composition on tailing factor (Y_2)

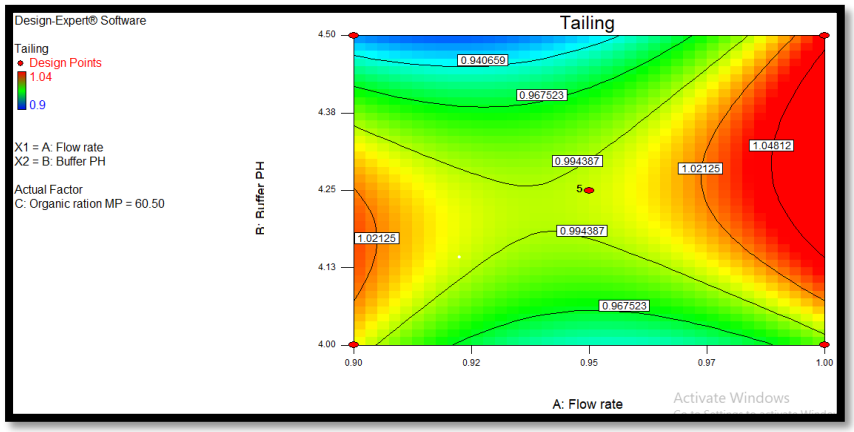

Figure 3: Overlay plot illustrating tailing factor (Y_2) within the MODR for SLM and DOM, confirming optimal operating region.

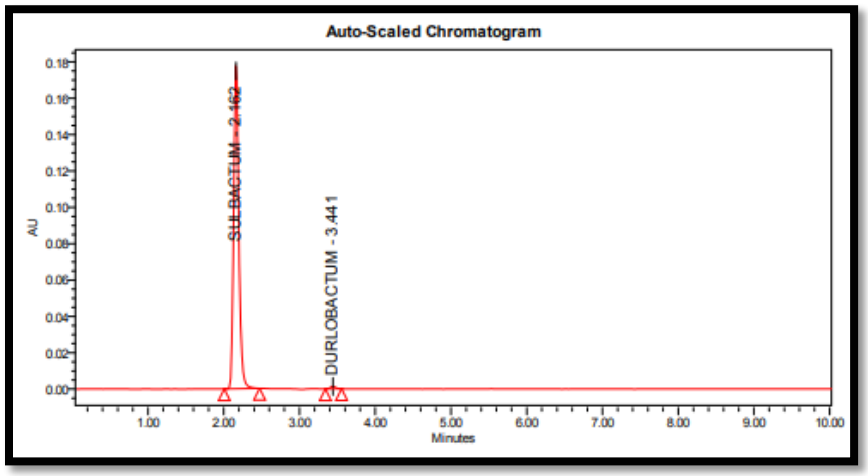

Figure 4: Representative chromatogram of SLM and DOM demonstrating system suitability

Table 1: System suitability results for SLM and DOM.

Analytes	Rt (min)	Area (µV·s)	Height (µV)	USP Tailing	USP Plates
SLM	2.162	18,895	1,109	1.00	5,797
DOM	3.441	789,931	22,645	0.85	2,357

Acceptance met: Resolution ≥ 2 between analytes (visual confirmation), T ≤ 2, and N ≥ 2000.

Table 2: Linearity data for SLM and DOM

Level	Conc. (µg·mL⁻¹)	Area (DOM)	Area (SLM)
1	10	6,299	273,312
2	20	12,599	526,625
3	30	18,899	789,938
4	40	25,198	1,053,250
5	50	32,498	1,316,563

4.3. Method operable design region (MODR) and selected operating point

The design space (MODR) was established by applying a desirability function to simultaneously maximize resolution (Y_1) and minimize tailing (Y_2). The optimization suggested that flow rate of 1.0 mL·min⁻¹, pH between 4.25–4.50, and organic phase ~65% provided the most desirable operating conditions.

From a practical and chemical standpoint, the buffer pH was finalized at 3.5 (formic acid system) to ensure reproducibility and solution stability. Experimental confirmation under these conditions demonstrated resolution ≥ 2.0 between SLM and DOM, with tailing factor ≤ 1.2, thus meeting the Analytical Target Profile (ATP).

4.4. System suitability and chromatography

System suitability was assessed using the optimized chromatographic conditions. The chromatogram (**Figure 3,Figure 4**) demonstrated baseline separation of SLM and DOM with no interference from excipients or diluents.

Table 1 presents the observed system suitability parameters. The retention times were 2.162 min (SLM) and 3.441 min (DOM). Both analytes exhibited sharp peaks with tailing factors ≤ 1.0 for SLM and 0.85 for DOM. The theoretical plates were well above 2000 for both compounds (SLM: 5797; DOM: 2357). The acceptance criteria of resolution ≥ 2.0, T ≤ 2.0, and N ≥ 2000 were satisfied, confirming system performance.

4.5. Linearity

Linearity was evaluated over the concentration range 10–50 µg·mL⁻¹ for both analytes. The calibration data (**Table 2)** demonstrated a strong linear response with regression coefficients (R^2) of 0.999 for both SLM and DOM.

The regression equations were:

SLM: y = 649.97x + 400.5 (R^2 = 0.999)

DOM: y = 26,131x + 7,999.5 (R^2 = 0.999)

The calibration plots (**Figure 5,Figure 6**) confirmed excellent correlation between concentration and peak area.

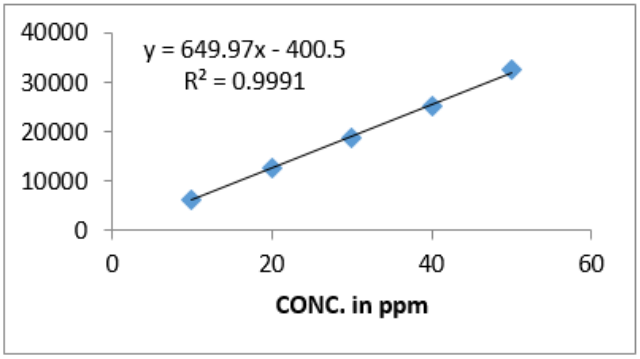

Figure 5: Calibration graph for SLM

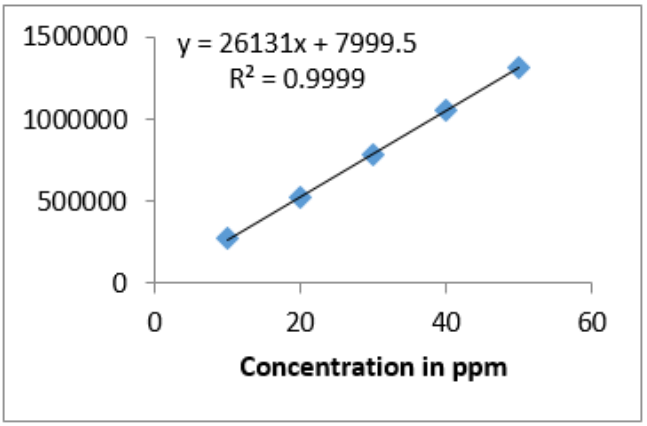

Figure 6: Calibration graph for DOM

4.6. Precision and intermediate precision

Repeatability (intra-day precision) and intermediate precision (inter-day/system) were assessed at working concentration (n=6).

For SLM, %RSD was 0.8, and for DOM, %RSD was 0.9. All values were within acceptance criteria (≤ 2%). This confirmed the method's high repeatability and reproducibility across systems and days.

Table 3: Precision and Intermediate Precision for SLM and DOM

Injection	Precision for SLM and DOM		Intermediate precision for SLM and DOM	
	Area	Area	Area	Area
Injection-1	7970152	16726	16726	7970152
Injection-2	8065041	16157	16557	8065041
Injection-3	7899251	16878	16678	7899251
Injection-4	7842995	16504	16514	7842995
Injection-5	7926488	16948	16928	7926488
Injection-6	7951230	16631	16631	7951230
Average	7942526	16640.67	16672.33	7942526
S. D	74679.64	286.6243	147.2123	74679.64
%RSD	0.94	1.72	0.8	0.9
S. D= Standard Deviation				

Table 4: Recovery results for SLM and DOM.

Level	Added (mg)	Found (mg)	% Recovery
50% (SLM)	12.5	12.2	97.6
100% (SLM)	25.0	24.5	98.0
150% (SLM)	37.5	37.1	98.9
50% (DOM)	12.5	12.2	97.6
100% (DOM)	25.0	24.5	98.0
150% (DOM)	37.5	37.1	98.9

Table 5: Effect of flow rate and organic composition variation on system suitability

Variation	SLM Plates	SLM Tailing	DOM Plates	DOM Tailing
Flow rate variation (mL·min⁻¹)				
0.8	5,721	1.10	2,349	1.01
1.0	5,799	1.20	2,351	0.89
1.2	5,793	0.98	2,345	0.96
Organic composition				
−10%	5,721	1.10	2,349	1.01
Actual	5,799	1.20	2,351	0.89
+10%	5,793	0.98	2,345	0.96

4.7. Accuracy (recovery)

Accuracy was determined by recovery studies at 50%, 100%, and 150% levels (Table 4). Recoveries ranged from 97.6% to 98.9%, with mean recovery of 98.1% for both SLM and DOM. These values were within the acceptance criteria of 98–102%, demonstrating excellent accuracy.

4.8. Sensitivity (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios of approximately 3:1 and 10:1, respectively.

SLM: LOD = 0.38 μg·mL⁻¹; LOQ = 1.2 μg·mL⁻¹

DOM: LOD = 0.08 μg·mL⁻¹; LOQ = 0.2 μg·mL⁻¹

These values indicate the method's high sensitivity for both analytes.

4.9. Robustness

Robustness was assessed by deliberate variations in flow rate (±0.2 mL·min⁻¹) and organic phase composition (±10%). Results (**Table 5)** showed negligible changes in system suitability parameters. Tailing factors remained between 0.89–1.20, and plate counts exceeded 2000 for DOM and 5700 for SLM. The assay remained unaffected, confirming method robustness.

5. Discussion

The application of the Analytical Quality by Design (AQbD) framework provided a structured and scientific basis for HPLC method development. Risk assessment and DoE analysis highlighted that organic proportion was the dominant driver of chromatographic resolution, followed by flow rate and key interactions (AB and BC). The significance of curvature terms in the tailing model underscores the importance of avoiding extreme conditions in CMPs,

consistent with a parabolic response surface at the boundaries of the MODR. The selected operating point (65% methanol, pH 3.5, flow rate 1.0 mL·min⁻¹) achieved an optimal balance of high resolution and low tailing, while maintaining short run times (retention time < 3.5 min for DOM), thereby enabling high-throughput quality control.

Validation studies confirmed that the method meets ICH Q2(R1) requirements. Linearity was demonstrated up to 50 µg·mL⁻¹ with $R^2 = 0.999$ for both analytes, precision and intermediate precision results were consistently ≤ 2%, and accuracy was within 98–102% across three recovery levels. Sensitivity studies established LOD and LOQ values appropriate for assay applications, and robustness testing demonstrated tolerance to deliberate variations in flow rate and organic proportion. Furthermore, system suitability was consistently met, verifying column performance and overall chromatographic health.

The method employed a compact 3µm particle C18 column, providing sufficient efficiency at moderate backpressure, which ensures compatibility with standard QC HPLC systems. Compared with published methods that utilize alternative stationary phases, phosphate buffers, or UPLC platforms,15-17 the present approach is advantageous in its use of readily available reagents (formic acid as aqueous modifier) and simple UV detection at 220 nm. This supports the goals of method simplicity, transferability, and cost-effectiveness, making the method accessible to a wide range of laboratories. Importantly, the BBD-derived MODR and the defined control strategy provide documented assurance of performance, aligning with regulatory expectations and facilitating lifecycle management.

6. Limitation

A limitation of this study is that the method validation focused primarily on assay performance. To extend its utility as a stability-indicating method, future work should include forced degradation studies under acid, base, oxidative, thermal, and photolytic conditions, in line with ICH Q1A(R2). Additionally, the use of volatile components in the mobile phase supports LC–MS compatibility, which can be employed for orthogonal specificity confirmation if required.

Overall, this study demonstrates that a QbD-driven approach not only yields a robust and reproducible HPLC method for simultaneous estimation of SLM and DOM, but also provides a regulatory-compliant framework that ensures long-term method reliability and flexibility.

7. Conclusion

A robust and reliable RP-HPLC method for the simultaneous estimation of sulbactam (SLM) and durlobactam (DOM) in pharmaceutical dosage forms was successfully developed and validated using the Analytical Quality by Design (AQbD) framework. Risk assessment and DoE studies identified organic proportion and flow rate as critical drivers of chromatographic performance, and the optimized operating point (65% methanol, pH 3.5, 1.0 mL·min⁻¹) ensured resolution ≥ 2.0, tailing ≤ 1.2, and short run times.

Method validation demonstrated compliance with ICH Q2(R1) criteria, with excellent linearity ($R^2 = 0.999$), precision (%RSD < 2%), accuracy within 98–102%, suitable sensitivity (LOD/LOQ), and robustness against small deliberate variations. System suitability parameters consistently met acceptance limits, confirming method reliability.

Compared with existing literature, the present method offers advantages in simplicity, reproducibility, and transferability, using readily available reagents and conventional UV detection. The BBD-derived MODR and defined control strategy provide documented assurance of performance, supporting regulatory acceptance and lifecycle management.

This method is therefore well suited for routine quality control and assay testing of SLM and DOM dosage forms. Future work should extend the method to forced degradation and peak purity studies to establish full stability-indicating capability.

8. Conflict of Interest

None declared.

9. Source of Funding

None.

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Cite this article: Kancharana I, G Divya, KEV Nagoji, Krishna K, Manju D, Sireesha I.QbD approach to HPLC method development and validation of the simultaneous estimation of sulbactam and durlobactam in pharmaceutical dosage form *Pharmacist*. 2025;5(1):6–13