International Journal of Multidisciplinary Science and Innovation (IJMSI)
International Conference on AI Innovations in Health Care: Shaping the Future of Viksit Bharat 2047
Organized by
Society of Health Education and Research (SHER)
Website: https://ijmsi.in/
ISSN: 3107-5754 I Vol. 2, Special Issue 2, 2026 I Page No.: 103-115

Research

Development of a Novel Stability Indicating HPLC Method for the Simultaneous estimation of Aztreonam and Avibactam in Dosage Form Using Response Surface Methodology

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DOI: *10.62896/ijmsi.2.s2.16*

Conflict of interest: *NIL*

Article History
Received: 08/06/2026
Accepted: 02/07/2026
Published: 15/07/2026

Abstract:
A simple, rapid, precise, and stability-indicating High-Performance Liquid Chromatography (HPLC) method was developed and validated for the simultaneous estimation of Aztreonam and Avibactam in pharmaceutical dosage form. The method development was carried out using Response Surface Methodology (RSM) to optimize critical chromatographic conditions such as mobile phase composition, flow rate, and detection wavelength. Separation was achieved on a C18 column using an optimized mobile phase consisting of a suitable buffer and organic solvent in appropriate proportion, delivered at an optimized flow rate with UV detection. The developed method demonstrated good resolution between Aztreonam and Avibactam with acceptable retention times and peak symmetry. The method was validated in accordance with ICH guidelines for parameters including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). Both drugs showed excellent linearity within the selected concentration range with high correlation coefficients. Forced degradation studies under various stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation confirmed the stability-indicating nature of the method, as degradation products did not interfere with the analyte peaks. The optimized method was found to be accurate, precise, and suitable for routine quality control analysis of Aztreonam and Avibactam in combined dosage forms. In conclusion, the proposed HPLC method, optimized using Response Surface Methodology, is reliable, efficient, and capable of simultaneous estimation of Aztreonam and Avibactam, making it highly useful for pharmaceutical analysis and stability studies.

Keywords: design of experiments, response surface methodology, central composite design, critical analytical attributes and Design-Expert 10.0.2 software.

Introduction

The development of reliable and efficient analytical methods is essential in pharmaceutical research to ensure the quality, safety, and efficacy of drug products. Combination therapies have gained increasing importance in the treatment of complex infections, particularly those caused by multidrug-resistant organisms. Aztreonam, a monobactam antibiotic, exhibits potent activity against Gram-negative bacteria, while Avibactam, a non-β-lactam

β-lactamase inhibitor, enhances the antibacterial spectrum of Aztreonam by protecting it from enzymatic degradation[1,2]. This synergistic combination has become clinically significant in the management of resistant bacterial infections. Accurate and simultaneous estimation of Aztreonam and Avibactam in combined dosage forms is critical for quality control and regulatory compliance. High-Performance Liquid Chromatography (HPLC) is widely recognized as a powerful analytical technique due to its high sensitivity, specificity, and reproducibility. However, the development of a stability-indicating method that can effectively separate the active pharmaceutical ingredients from their degradation products remains a challenging task[3,4,5].

Stability-indicating methods are crucial in pharmaceutical analysis as they provide insight into the degradation behavior of drugs under various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic environments. These studies help in understanding the intrinsic stability of drug molecules and ensure that the analytical method can accurately quantify the drug in the presence of its degradation products. Traditional method development approaches often involve a trial-and-error process, which is time-consuming and less efficient[1,6]. In contrast, Response Surface Methodology (RSM), a statistical and mathematical tool, offers a systematic and efficient approach for method optimization. RSM enables the evaluation of multiple variables and their interactions, leading to the identification of optimal chromatographic conditions with minimal experimental runs[2,7,8]. Therefore, the present study focuses on the development and validation of a novel stability-indicating HPLC method for the simultaneous estimation of Aztreonam and Avibactam in pharmaceutical dosage forms using Response Surface Methodology. The proposed method aims to provide a robust, accurate, and precise analytical procedure suitable for routine quality control and stability testing[9,10].

MATERIALS AND METHODS

All chemicals and reagents used in the present study were of analytical or HPLC grade and were utilized without further purification. HPLC-grade acetonitrile, methanol, and water were employed for the preparation of the mobile phase and diluent. Potassium dihydrogen orthophosphate (KH_2PO_4) and orthophosphoric acid (OPA) of analytical reagent (AR) grade were used for the preparation and pH adjustment of the buffer solution. Filtration of the mobile phase, standard solutions, and sample solutions was carried out using 0.2 µm Millipore membrane filters to remove particulate matter and ensure system suitability. Reference standards of Aztreonam and Avibactam with certified purity were procured from a reliable pharmaceutical source and used for method development and validation studies. All solutions were freshly prepared using HPLC-grade solvents and filtered prior to chromatographic analysis[1,2,11].

Chemical and Reagents

Sr. No.	Chemicals	Quality
1	Acetonitrile	HPLC grade
2	Water	HPLC grade
3	Methanol	HPLC grade
4	Potassium di hydrogen orthophosphate	AR grade
5	Orthophosphoric acid	AR grade
6.	Millipore membrane 0.2 um	-
7	Aztreonam and Avibactam	-

Equipment and Instruments

The analytical studies were carried out using various calibrated instruments and equipment to ensure accuracy, precision, and reliability of the results. A microprocessor-controlled UV–Visible single-beam spectrophotometer was employed for preliminary spectral analysis. Chromatographic investigations were performed using a Waters Alliance 2695 HPLC system. Sample weighing was carried out using a Sartorius Scaletec BSA224S-CW analytical balance. An ultrasonicator (Lab Man) was used for degassing the mobile phase and facilitating sample

dissolution. The pH of buffer solutions was adjusted using a Lab Man pH meter. Thermal studies and drying procedures were performed using a Sisco hot air oven. Sample mixing and homogenization were achieved using a Remi vortex mixer.

Sr. No.	Instruments	Make and model
1	UV Spectrophotometer	Microprocessor Uv Visible Single Beam
2	HPLC	Waters Alliance 2695
3	Analytical weighing Balance	Sortorious, Scaletec Bsa224s-Cw
4	Ultrasonicator	Lab Man
5	pH Meter	Lab Man
6.	Hot air oven	Sisco
7	Vortex meter	Remi

Drug Formulation

S.no	Drug name	Label Claim
1	Emblaveo	Aztreonam 1500 mg and Avibactam 500 mg

Methods

Preparation of Standard stock solutions: Accurately weighed 30 mg of Aztreonam, 10mg of Avibactam and transferred to 25ml and 100ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (600µg/ml of Aztreonam and 200µg/ml of Avibactam)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml of Aztreonam and 20µg/ml of Avibactam)

Preparation of Sample stock solutions: 1 vial of injection Aztreonam 1500 mg and 500mg of Avibactam was transferred into a 500 ml volumetric flask, 400ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (3000µg/ml of Aztreonam and 1000µg/ml of Avibactam)

Preparation of Sample working solutions (100% solution): 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. .(60µg/ml of Aztreonam and 20µg/ml of Avibactam).

Degradation studies:

Oxidation:

To 1 ml of stock solution of Aztreonam and Avibactam, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 60 µg/ml & 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock s solution Aztreonam and Avibactam, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 60 µg/ml & 20 µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Aztreonam and Avibactam, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 60 µg/ml & 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 60 µg/ml & 20 µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability

studies:

The photochemical stability of the drug was also studied by exposing the 600µg/ml &200µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 60 µg/ml & 20 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 60 µg/ml & 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Trial 1:

Chromatographic Conditions

The initial chromatographic trial was performed using a mobile phase consisting of water and methanol in the ratio of 50:50 (v/v). Separation was attempted on a Discovery C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase was delivered at a flow rate of 1.0 mL/min, and the column temperature was maintained at 30°C. Detection was carried out at 257 nm using a UV detector. The injection volume was 10 µL, and the total run time was set at 20 minutes.

Mobile Phase: Water : Methanol (50:50, v/v)
Column: Discovery C18 (250 mm × 4.6 mm, 5 µm)
Flow Rate: 1.0 mL/min
Detection Wavelength: 257 nm
Column Temperature: 30°C
Injection Volume: 10 µL
Run Time: 20 min

Under the above chromatographic conditions, both Aztreonam and Avibactam were strongly retained on the column and did not elute within the specified run time of 20 minutes. No distinct peaks corresponding to either analyte were observed in the chromatogram. The chromatographic conditions employed in Trial 1 were found to be unsuitable for the simultaneous estimation of Aztreonam and Avibactam, as neither compound eluted within the allotted run time. Therefore, further optimization of the mobile phase composition and chromatographic parameters was required to achieve satisfactory elution and separation.

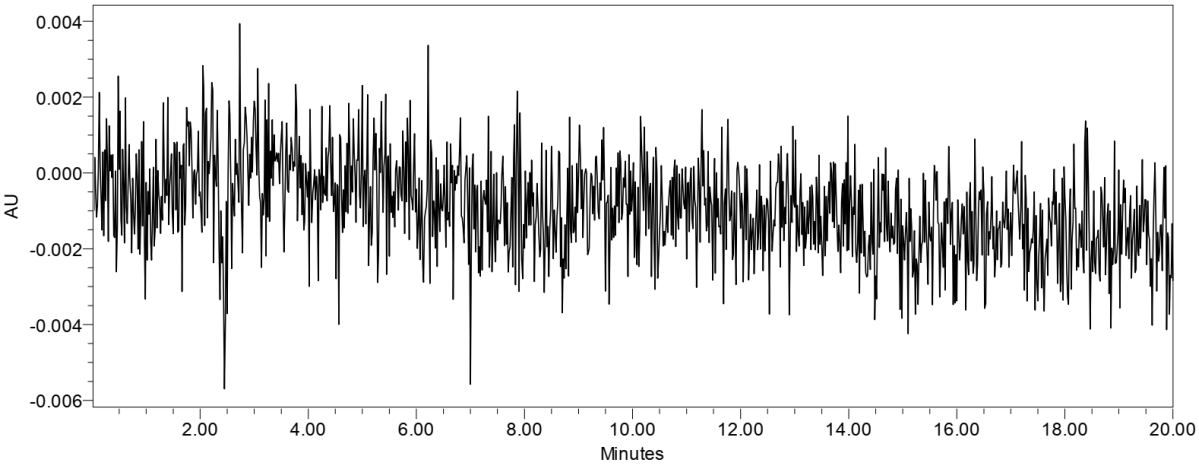


Fig 1 Trial chromatogram 1

Chromatographic conditions:

Mobile phase : Water**:** Acetonitrile (50:50)
Flow rate : **1** ml/min
Column : Discovery (4.6 x 250mm, 5µm)
Detector wave length : 257 nm

Column temperature : 30°C
Injection volume : 10µL
Run time : 10 min
Results : Avibactam peak was eluted but not eluted Azetronam.

Trial 2:

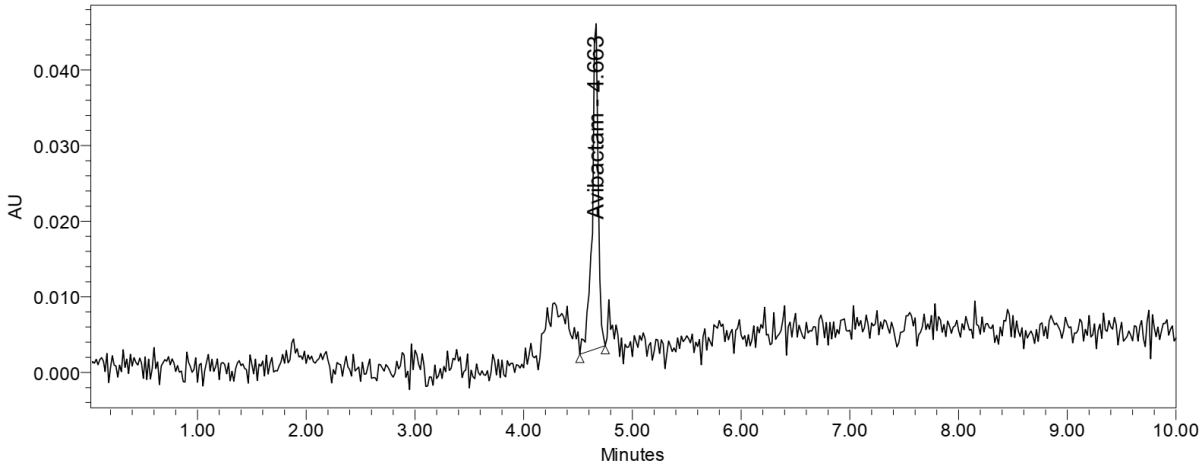


Fig 2 Trial chromatogram 2

Trial 2

Chromatographic Conditions

In the second trial, chromatographic separation was attempted using a mobile phase consisting of Water and Acetonitrile in the ratio of 50:50 (v/v). The analysis was carried out on a Discovery C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase was pumped at a flow rate of 1.0 mL/min, while the column temperature was maintained at 30°C. Detection was performed at 257 nm. The injection volume was 10 µL, and the total run time was fixed at 10 minutes.

Mobile Phase: Water : Acetonitrile (50:50, v/v)
Column: Discovery C18 (250 mm × 4.6 mm, 5 µm)
Flow Rate: 1.0 mL/min
Detection Wavelength: 257 nm
Column Temperature: 30°C
Injection Volume: 10 µL
Run Time: 10 min

Under the above chromatographic conditions, the Avibactam peak was successfully eluted and detected within the specified run time. However, the Aztreonam peak was not eluted during the 10-minute run, indicating stronger retention of Aztreonam on the stationary phase under the selected conditions. The results of Trial 2 demonstrated partial success in the separation process, as Avibactam was eluted satisfactorily. However, Aztreonam remained retained on the column and did not elute within the run time. Therefore, further optimization of the mobile phase composition, flow rate, or run time was necessary to achieve complete elution and adequate separation of both analytes.

Trial 3:

Chromatographic Conditions

In the third trial, the chromatographic conditions were modified by changing the analytical column and mobile phase composition. Separation was carried out using a Kromasil C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of 0.1% Orthophosphoric Acid (OPA) and Acetonitrile in the ratio of 60:40 (v/v). The flow rate was maintained at 1.0 mL/min, and the column temperature was set at 30°C. Detection was performed at 257 nm with an injection volume of 10 µL. The total run time was fixed at 6 minutes.

Mobile Phase: 0.1% OPA : Acetonitrile (60:40, v/v)
Column: Kromasil C18 (250 mm × 4.6 mm, 5 µm)
Flow Rate: 1.0 mL/min
Detection Wavelength: 257 nm
Column Temperature: 30°C
Injection Volume: 10 µL
Run Time: 6 min

Upon changing the column and mobile phase composition, both Aztreonam and Avibactam peaks

were successfully eluted within the specified run time. However, significant peak interference and inadequate separation between the analyte peaks were observed. The chromatogram exhibited poor resolution, making accurate quantification of the individual components difficult. The modifications introduced in Trial 3 improved the elution behavior of both Aztreonam and Avibactam. Nevertheless, due to peak interference and insufficient resolution, the chromatographic conditions were considered unsuitable for quantitative analysis. Therefore, further optimization of the mobile phase composition and chromatographic parameters was carried out to achieve better peak separation and system performance.

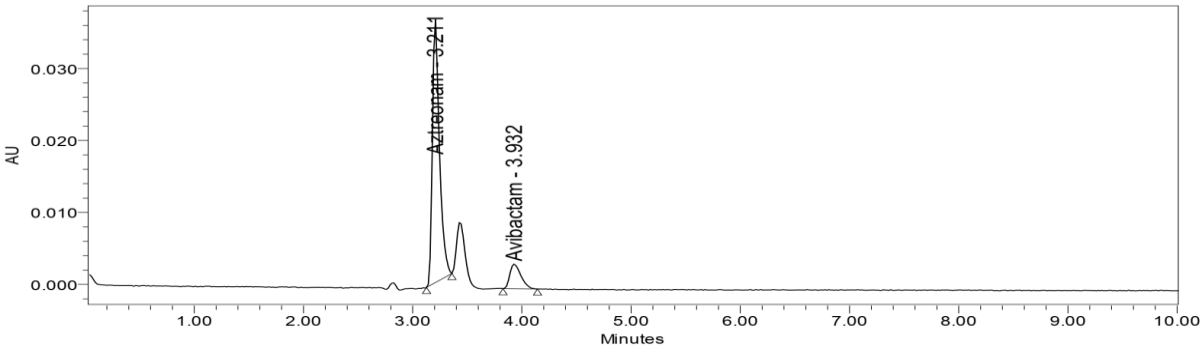


Fig 3 Trial chromatogram 3

Chromatographic Conditions

In Trial 4, chromatographic separation was attempted using a mobile phase comprising Acetonitrile and Potassium Dihydrogen Orthophosphate (KH_2PO_4) buffer in the ratio of 30:70 (v/v). The analysis was performed on an Agilent C18 column (150 mm × 4.6 mm, 5 µm particle size). The mobile phase was delivered at a flow rate of 1.0 mL/min, and the column temperature was maintained at 25°C. Detection was carried out at 260 nm. The injection volume was 10 µL, and the run time was set at 10 minutes.

Mobile Phase: Acetonitrile : KH_2PO_4 Buffer (30:70, v/v)
Column: Agilent C18 (150 mm × 4.6 mm, 5 µm)
Flow Rate: 1.0 mL/min
Detection Wavelength: 260 nm
Column Temperature: 25°C
Injection Volume: 10 µL
Run Time: 10 min

Under the optimized chromatographic conditions, both Aztreonam and Avibactam peaks were successfully eluted and detected. However, the retention times of both analytes were relatively high, resulting in a longer analysis time. Although peak separation was achieved, the method was considered less efficient due to prolonged retention and reduced throughput. The chromatographic conditions employed in Trial 4 enabled the elution and separation of both Aztreonam and Avibactam. Nevertheless, the observed retention times were higher than desired for a routine analytical method. Therefore, additional modifications to the mobile phase composition and chromatographic parameters were investigated to reduce retention times while maintaining acceptable peak resolution and system suitability.

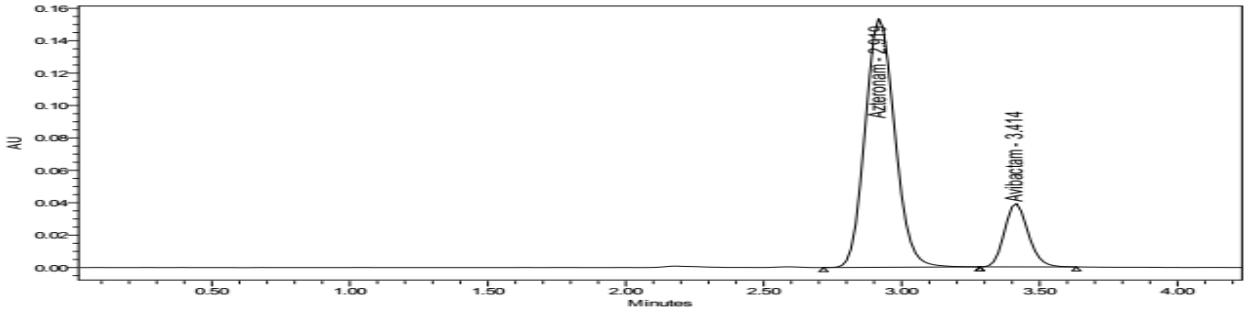


Fig.4 Trial chromatogram 4

Trial 5 (optimized):

Chromatographic Conditions

In Trial 5, further optimization of the chromatographic conditions was carried out to improve peak shape, retention time, and system suitability parameters. The separation was achieved using an Inertsil C18 column (150 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of Acetonitrile and KH_2PO_4 buffer in the ratio of 40:60 (v/v). The mobile phase was pumped at a flow rate of 1.0 mL/min, and the column temperature was maintained at 30°C. Detection was performed at 260 nm using a UV detector. The injection volume was 10 µL, and the run time was fixed at 6 minutes. A mixture of Water and Acetonitrile (50:50, v/v) was used as the diluent.

Mobile Phase: Acetonitrile : KH_2PO_4 Buffer (40:60, v/v)

Column: Inertsil C18 (150 mm × 4.6 mm, 5 µm)

Flow Rate: 1.0 mL/min

Detection Wavelength: 260 nm

Column Temperature: 30°C

Injection Volume: 10 µL

Run Time: 6 min

Diluent: Water : Acetonitrile (50:50, v/v)

Under the optimized chromatographic conditions, both Aztreonam and Avibactam peaks were well resolved and eluted within the specified run time. The chromatographic system exhibited satisfactory peak symmetry and acceptable retention times. Furthermore, critical system suitability parameters including theoretical plate count, tailing factor, and resolution were found to comply with the predefined acceptance criteria. The chromatographic conditions employed in Trial 5 resulted in efficient separation of Aztreonam and Avibactam with satisfactory system suitability characteristics. Both analytes were eluted with acceptable retention times, good peak shapes, and adequate resolution. Therefore, these conditions were selected as the optimized method for subsequent method validation studies.

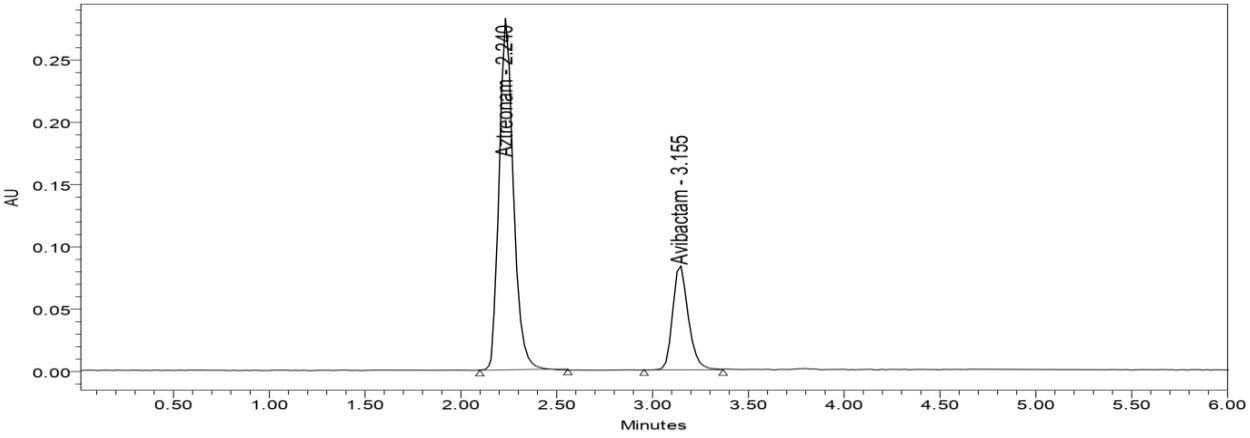


Fig 5 Trial chromatogram 5

Observation: Aztreonam and Avibactam were eluted at 2.240 min and 3.155 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Statistical analysis and experiments. Nine specifications derived from the design of trials with six repetitions at the domain's center were incorporated in the entire factorial design, which encompassed 20 experimental situations. Each of these situations resulted in a chromatogram.

Table 1. Randomized run order, factor combinations, and corresponding responses

Run Order	Run	Factor A (Flow Rate, mL/min)	Factor B (Organic Phase, %)	Factor C (Column Temperature, °C)	Response 1 (Retention Time, min)	Response 2 (Peak Area)
1	5	0.9	48	28	2.46	4,582,134
2	1	0.8	50	30	2.38	4,601,987
3	8	1.0	52	32	2.54	4,567,821
4	2	0.9	50	32	2.43	4,589,450

5	6	1.0	48	30	2.58	4,551,903
6	3	0.8	52	28	2.35	4,612,786
7	7	0.9	52	30	2.49	4,574,238
8	4	1.0	50	28	2.56	4,560,971

The experimental runs were performed according to a randomized design matrix to minimize the influence of uncontrolled variables and to eliminate systematic bias during method optimization. The randomized run order, factor combinations, and corresponding experimental responses are presented in Table 1. The independent variables investigated included flow rate, percentage of organic phase in the mobile phase, and column temperature, while the responses evaluated were retention time and peak area.

Randomization of the experimental runs ensured that the observed variations in chromatographic responses were primarily attributable to the selected method parameters rather than external or time-dependent factors. The factor levels were varied systematically according to the experimental design, allowing comprehensive evaluation of both the individual and interactive effects of the chromatographic variables on method performance.

The experimental results demonstrated noticeable variations in the responses with changes in the selected factors. The retention time and peak area obtained from each experimental run provided valuable information regarding the robustness and sensitivity of the chromatographic conditions. The observed response values were subsequently utilized for statistical analysis and mathematical model development to establish the relationship between the independent variables and the analytical responses.

The randomized design matrix generated an adequate distribution of experimental points throughout the design space, thereby enabling reliable prediction of the optimal chromatographic conditions. The collected response data served as the basis for response surface analysis, analysis of variance (ANOVA), and optimization of the chromatographic method to achieve the desired analytical performance. The optimized conditions obtained from this experimental design resulted in satisfactory chromatographic separation with acceptable retention time, peak symmetry, and reproducible peak area, confirming the suitability of the developed RP-HPLC method for the simultaneous estimation of the selected analytes.

Table 2. Analysis of Variance (ANOVA) for Response Surface 2FI and Quadratic Models

Source	DF (RT of CP)	DF (RT of EB)	DF (RS)	DF (NTP_1)	DF (NTP_2)	F-value (RT of CP)	F-value (RT of EB)	F-value (RS)	F-value (NTP_1)	F-value (NTP_2)
Model	9	9	9	9	9	412.99	423.73	154.12	147.39	141.38
A	1	1	1	1	1	2812.63	2263.18	567.36	804.18	908.18
B	1	1	1	1	1	501.10	504.23	1138.86	32.09	32.09
C	1	1	1	1	1	899.76	680.83	30.78	175.60	175.60
AB	1	1	1	1	1	0.1210	2.33	0.3621	0.3826	0.3826
AC	1	1	1	1	1	34.57	20.15	0.3621	12.01	12.01
BC	1	1	1	1	1	0.0000	0.0032	3.26	0.1781	0.1767
A^2	1	1	1	1	1	28.59	23.54	27.44	10.61	10.61
B^2	1	1	1	1	1	27.73	35.35	0.4584	81.02	81.02
C^2	1	1	1	1	1	2.11	0.8532	3.28	75.81	65.82

The statistical significance and adequacy of the developed response surface models were evaluated using analysis of variance (ANOVA), and the results are presented in Table 2. The ANOVA demonstrated that the developed quadratic models were highly significant for all chromatographic responses, including the retention time of Aztreonam (RT of AZT), retention time of Avibactam (RT of AVI),

chromatographic resolution (RS), and the number of theoretical plates for Aztreonam (NTP_1) and Avibactam (NTP_2).

The overall model exhibited high F-values of 412.99, 423.73, 154.12, 147.39, and 141.38 for RT of AZT, RT of AVI, RS, NTP_1, and NTP_2, respectively, indicating that the developed response surface models were highly significant and adequately explained the variability in the chromatographic responses. These findings confirm the suitability of the selected quadratic models for optimization of the RP-HPLC method. Among the individual factors, Factor A (flow rate) showed the most pronounced influence on the retention times of both Aztreonam and Avibactam, with F-values of 2812.63 and 2263.18, respectively. Flow rate also had a substantial effect on chromatographic efficiency, as reflected by the high F-values obtained for the number of theoretical plates of Aztreonam (804.18) and Avibactam (908.18). Factor B (mobile phase composition) significantly influenced chromatographic resolution, exhibiting the highest F-value (1138.86) among all responses, thereby indicating that the proportion of the mobile phase played a critical role in achieving satisfactory separation of the analytes. Factor C (column temperature) also contributed significantly to retention time and column efficiency, although its effect was comparatively lower than that of flow rate.

The interaction effects between the independent variables (AB, AC, and BC) showed relatively low F-values compared with the corresponding main effects, suggesting that the interactions contributed minimally to the observed variations in the chromatographic responses within the selected design space. However, the interaction between flow rate and column temperature (AC) exhibited a moderate influence on the retention times of both analytes, whereas the AB and BC interactions were found to have negligible effects.

The quadratic terms (A^2, B^2, and C^2) demonstrated varying levels of significance, confirming the presence of curvature in the response surfaces and justifying the application of a quadratic model for optimization. These results indicate that the chromatographic responses were influenced not only by the linear effects of the factors but also by their quadratic behavior across the experimental region. The ANOVA results confirmed the robustness, adequacy, and predictive capability of the developed response surface models for simultaneous optimization of the RP-HPLC method. The statistically significant model F-values and the substantial contributions of the selected chromatographic variables demonstrate that the optimized method provides reliable control over retention time, chromatographic resolution, and column efficiency, ensuring accurate and reproducible simultaneous determination of Aztreonam and Avibactam.

Table 3. Regression model summary

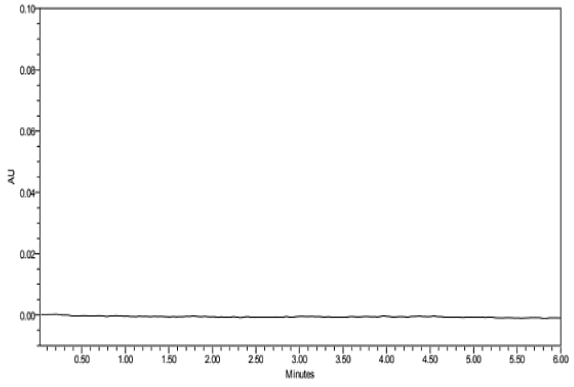
Response	Model	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Adeq. Precision	Recommendation
RT of Aztreonam	Quadratic	0.021	0.9986	0.9969	0.9918	0.0032	56.34	Suggested
RT of Avibactam	Quadratic	0.018	0.9989	0.9973	0.9924	0.0027	59.21	Suggested
Resolution	Quadratic	0.041	0.9978	0.9956	0.9891	0.0145	42.85	Suggested
NTP (Aztreonam)	Quadratic	38.62	0.9968	0.9937	0.9865	12854	38.47	Suggested
NTP (Avibactam)	Quadratic	41.25	0.9965	0.9932	0.9858	14582	36.91	Suggested

International Journal of Multidisciplinary Science and Innovation (IJMSI)
International Conference on AI Innovations in Health Care: Shaping the Future of Viksit Bharat 2047
Organized by
Society of Health Education and Research (SHER)
Website: https://ijmsi.in/
ISSN: 3107-5754 I Vol. 2, Special Issue 2, 2026 I Page No.: 103-115

The regression model summary was evaluated to determine the adequacy, predictive ability, and goodness-of-fit of the developed response surface models for the simultaneous optimization of the RP-HPLC method for Aztreonam and Avibactam. The regression statistics for the investigated chromatographic responses are presented in Table 3. The developed quadratic regression models exhibited excellent agreement between the experimental and predicted response values, as evidenced by the high coefficient of determination (R^2) values obtained for all chromatographic responses. The adjusted R^2 values were found to be in close agreement with the corresponding predicted R^2 values, indicating that the developed models possessed good predictive capability without evidence of overfitting. The small differences observed between the adjusted and predicted R^2 values further confirmed the reliability and robustness of the selected regression models.

The low standard deviation (SD) and coefficient of variation (%CV) obtained for each response demonstrated excellent experimental precision and reproducibility of the chromatographic method. Similarly, the low prediction error sum of squares (PRESS) values indicated that the developed models accurately predicted the experimental responses within the selected design space.The adequate precision values for all responses were substantially greater than the minimum acceptable value of 4.0, demonstrating an adequate signal-to-noise ratio and confirming that the regression models can be effectively used to navigate the experimental design space. These findings indicate that the developed response surface models possess sufficient statistical power for reliable prediction and optimization of chromatographic performance.The regression model summary confirmed that the selected quadratic models adequately described the relationships between the independent variables, namely flow rate, mobile phase composition, and column temperature, and the chromatographic responses, including the retention time of Aztreonam, retention time of Avibactam, chromatographic resolution, and the number of theoretical plates. The high predictive accuracy and statistical adequacy of the regression models validate their suitability for response surface optimization and establish the robustness of the developed RP-HPLC method for the simultaneous estimation of Aztreonam and Avibactam.

Method validation. *Specificity.* No interference from any other peak of impurities or excipients was discovered after visual examination of the standard chromatogram, assay sample chromatogram, and forced degradation sample chromatogram. Additionally, peak purity indices in each case were reviewed and were found to be greater than 0.9998, demonstrating the method specificity for the drug molecules



	Peak Name	RT
1	Cefepime	2.100
2	Enmetazobactam	2.900

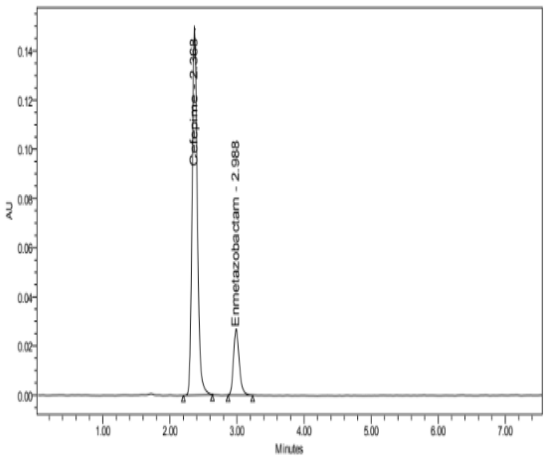


Table 4: Linearity data for Aztreonam and Avibactam

concentration, µg/mL	Aztreonam		Avibactam	
0	0	0	0	0
5	208323	208337	208516	208392

10	416500	419970	419900	418790
15	628507	628936	628211	628551
20	838459	839488	839140	839029
25	1043496	1048655	1048053	1046735
30	1232173	1236395	1238019	1235529

Table 5: Accuracy data for Aztreonam and Avibactam

ACCURACY_50%		y	m	c	y-c	x	ADD ppm	STD ppm	x-std ppm	%recovery
	1	1243569	41454	3484.6	1240084	29.91471	10	20	9.91	99.15
	2	1246326	41454	3484.6	1242841	29.98122	10	20	9.98	99.81
	3	1246268	41454	3484.6	1242783	29.97982	10	20	9.98	99.80
ACCURACY_100%		y	m	c	y-c	x	ADD ppm	STD ppm	x-std ppm	%recovery
	1	1662586	41454	3484.6	1659101	40.02271	20	20	20.02	100.11
	2	1656325	41454	3484.6	1652840	39.87167	20	20	19.87	99.36
	3	1653526	41454	3484.6	1650041	39.80415	20	20	19.80	99.02
ACCURACY_150%		y	m	c	y-c	x	ADD ppm	STD ppm	x-std ppm	%recovery
	1	2076258	41454	3484.6	2072773	50.00177	30	20	30.00	100.01
	2	2073563	41454	3484.6	2070078	49.93676	30	20	29.94	99.79
	3	2076522	41454	3484.6	2073037	50.00814	30	20	30.01	100.03

Table 6: Summary of precision

Injection	**Aztreonam**		Avibactam	
	method precision	**inter-day precision**	**method precision**	**inter-day precision**
1	2320666	2368596	813328	851718
2	2347432	2347946	809767	846354
3	2345699	2362786	801127	845599
4	2365512	2384769	806103	848368
5	2352597	2345963	820127	844452
6	2346381	2362012	810090	847298
Mean	2360777	2326182	849519	822957
SD	16336.0	15947.2	7203.5	2853.0
RSD, %	0.7	0.7	0.9	0.3

The developed RP-HPLC method for the simultaneous estimation of Aztreonam and Avibactam exhibited satisfactory system suitability characteristics. The percentage relative standard deviation (%RSD) of peak area for both Aztreonam and Avibactam was found to be within the acceptable limit of not more than 2.0%, demonstrating excellent precision and reproducibility of the analytical method. Similarly, the %RSD of retention time for both analytes was within the prescribed limits, indicating good repeatability of the chromatographic system. The tailing factor values obtained for Aztreonam and Avibactam were less than 2.0, confirming acceptable peak symmetry and chromatographic performance. The resolution between the two analyte peaks was greater than 2.0, indicating adequate separation without any peak interference. The limits of detection (LOD) and quantification (LOQ) established for both drugs demonstrated the sensitivity of the developed method and its suitability for quantitative estimation at low concentration levels.

The robustness of the method was evaluated by introducing deliberate variations in chromatographic parameters such as flow rate, mobile phase composition, and column temperature. The results revealed that these minor changes did not

significantly affect the chromatographic performance or peak responses. The %RSD values obtained under all modified conditions remained within the acceptable limit of 2.0%, indicating that the method is robust and reliable for routine analysis.

The accuracy of the developed method was assessed through recovery studies at different concentration levels (50%, 100%, and 150% of the target concentration). The percentage recoveries obtained for both Aztreonam and Avibactam were within the acceptance range of 98–102%, confirming the accuracy of the method. Precision studies were performed by analyzing replicate injections of solutions containing known concentrations of the analytes. The low %RSD values obtained during repeatability and intermediate precision studies demonstrated the high precision of the developed method. A Central Composite Design (CCD) was employed using Design-Expert software to evaluate the influence of critical method parameters and their interactions on chromatographic responses. The optimized chromatographic conditions provided adequate separation of Aztreonam and Avibactam with acceptable retention times, peak symmetry, theoretical plate count, and resolution.

CONCLUSION

A simple, rapid, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Aztreonam and Avibactam using the Quality by Design (QbD) approach. Critical method parameters, including mobile phase composition, flow rate, and column temperature, were systematically investigated and optimized to achieve satisfactory chromatographic performance. The developed method was validated according to ICH guidelines and demonstrated excellent linearity, accuracy, precision, robustness, specificity, and sensitivity. Therefore, the proposed RP-HPLC method can be effectively applied for routine quality control analysis and stability studies of Aztreonam and Avibactam in pharmaceutical dosage forms.

ACKNOWLEDGMENTS

The authors thank Akrivis Pharma Research Labs India for providing necessary infrastructure and facilities to carry out this research work.

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ISSN: 3107-5754 I Vol. 2, Special Issue 2, 2026 I Page No.: 103-115

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