Available online on 15.02.2026 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access **Research Article**

RP-HPLC Analytical Method Development and Validation of Avibactam and Aztreonam

Gangu Sreelatha*, Nandini Kolli, Arunabha Mallik

Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana, India

A B S T R A C T

A simple, rapid, precise, sensitive, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Avibactam and Aztreonamin pharmaceutical dosage form. Chromatographic separation of Avibactam and Aztreonamwas achieved on Waters Alliance-e2695by using Luna Phenyl Hexyl (250x 4.6mm, 5µ) column and the mobile phase containing Acetonitrile: 0.1% Formic acid in the ratio of 20:80% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 250nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factors for Avibactam and Aztreonam were NLT 2000 and should not be more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Avibactam and Aztreonam study of its stability.

Key words: Avibactam, Aztreonam, Luna Phenyl Hexyl, Acetonitrile, Formic Acid.

A R T I C L E I N F O: Received 20 Dec. 2025 ; Review Complete 16 Jan 2026 ; Accepted 02 Feb. 2026; Available online 15 Feb. 2026

Cite this article as:

Sreelatha G, Kolli N, Arunabha Mallik, RP-HPLC Analytical Method Development and Validation of Avibactam and Aztreonam, Asian Journal of Pharmaceutical Research and Development. 2026; 14(1):15-19, DOI: http://dx.doi.org/10.22270/ajprd.v14i1.1688

*Address for Correspondence:

Dr. Gangu Sreelatha, Associate Professor,Department of Pharmaceutical Analysis,Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad – 500 046, Telangana, India.

INTRODUCTION

HPLC is a liquid chromatographic separation technique conducted in high pressure where the sample (mixture constituents) is separated into its individual components by passing the sample present in a mobile phase (a flowing liquid) into a stationary phase (sorbents packed inside a column). Separation is due to the difference in affinity of individual components with mobile phase and stationary phase. The basic components are solvent delivering unit, sample injector, column, and a detector [1,2].

Avibactam is an inhibitor of beta-lactamases, which are enzymes that bacteria use to resist antibiotics. It is used to treat bacterial infections, particularly when combined with other antibiotics such as ceftazidime or aztreonam. Aztreonamis used to treat bacterial infections, doctors prescribe aztreonam, an antibiotic. To put it simply, it kills germs or at least stops them from multiplying.Aztreonam is a multi-system antibiotic that fights against bacterial infections [3-5].

During our literature survey we found out that there are many HPLC methods available for determination of Avibactam and Aztreonam both individually and in combination with other drugs. There are few LCMS/MS methods which are available for determination of Avibactam and Aztreonam. There is one bioanalytical assay also available for these drugs. Our developed method is more economical, eco-friendly, and sensitive compared to other available LCMS/MS, HPLC-MS/MS, HPLC-UV methods [6-9].

MATERIALS AND METHODS

Chemicals and reagents

Avibactam and Aztreonam (API) was obtained as a gift sample from Hetero LabsLtd., Hyderabad, Telangana.All the chemicals and reagents used were analytical grade. Methanol, acetonitrile and distil water used were of HPLC grade.

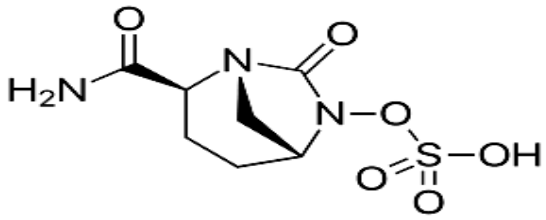

Figure 1: Chemical structure of avibactam

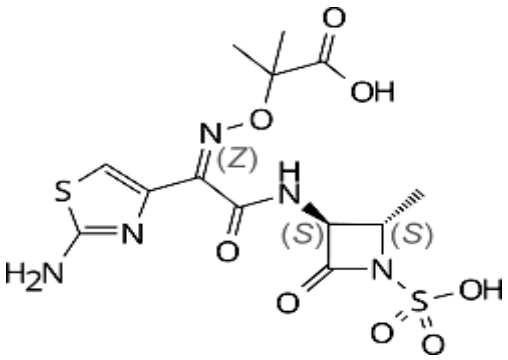

Figure 2: Chemical structure of aztreonam

System suitability

In accordance with ICH standards, all system suitability parameters were acceptable and within the specified range [10-12].

Analytical method validation

The method's accuracy, precision, specificity, and linearity range were all confirmed. Following ICH criteria, the method was validated.Avibactam had a retention duration of 2.254 minutes and Aztreonam of 3.536 minutes. At retention durations for these medications, we did not detect any interference peaks in the placebo and blank samples using this approach. Thus, it was claimed that this approach was precise.

Precision:

A homogeneous sample from a single batch should be analyzed six times for technique precision. This tells you whether the results of a procedure are stable for only one batch. Find the percentage RSD by running the sample six times.The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 100ppm of Avibactam, 50ppm of Aztreonam).

Linearity:

Fill a 10-milliliter volumetric flask to the mark with the same solvent after accurately weighing 10 mg of Avibactam and 5 mg of Aztreonam working standard. Add the diluent and sonicate until fully dissolved.Put each solution into the chromatographic apparatus and find its peak response.

Assay:

$$\%\ \text{Assay} = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{\text{Average weight}}{\text{Label Claim}} * \frac{P}{100} * 100$$

LOD and LOQ:

To determine the drug carry's detection and quantification limits, we employed the following equation, which was recommended by the worldwide conference on harmonization.

LOD = 3.3 X σ /S

LOQ = 10 X σ /S

Results for all the validation parameters are shown in the belowFigures and Tables.

Degradation studies:

Various degradation studies were conducted the results were found to be in acceptance range [13-18].

RESULTS AND DISCUSSION

Validated analytical methods are aimed for the determination of levetiracetam in API and its formulation. All the analytical validation parameters for the proposed method were determined according to ICH guidelines.

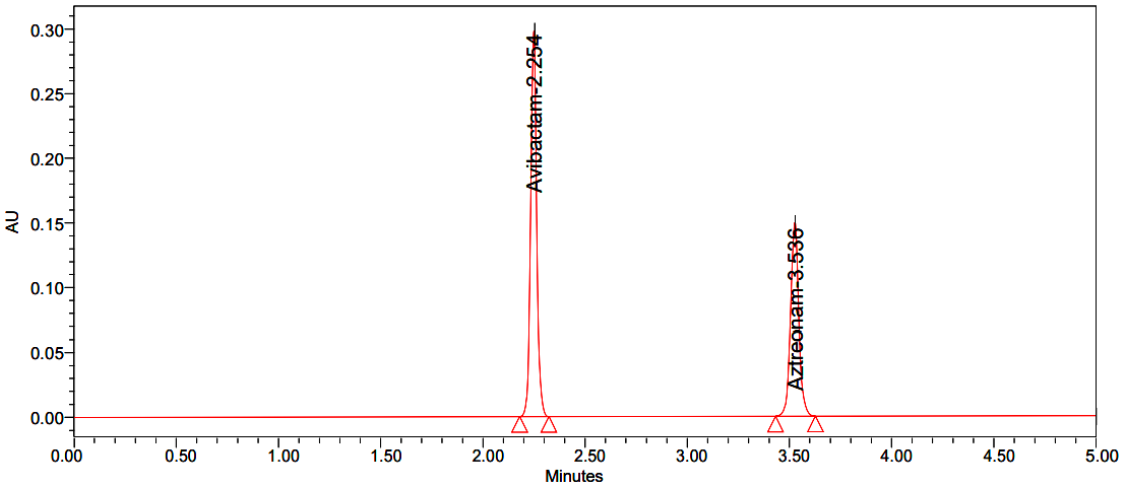

Figure 3: Optimized Chromatogram

Table 1: Drug Profile- Avibactam

IUPAC Name	[(2*S*,5*R*)-2-Carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulphate
Molecular Formula	$C_7H_{11}N_3O_6S$
Molecular Weight	265.25 g/mol

Table 2: Drug Profile- Aztreonam

IUPAC Name	2-{[(1*Z*)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(2*S*,3*S*)-2 methyl-4-oxo-1-sulfoazetidin-3-yl]amino}-2 oxoethylidene]amino}oxy-2-methylpropanoic acid
Molecular Formula	$C_{13}H_{17}N_5O_8S_2$
Molecular Weight	435.4 g/mol

Method validation

The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Table 3: System suitability parameters for Avibactam & Aztreonam

S. No	Parameter	Avibactam	Aztreonam
1	RT	2.254	3.536
2	Theoretical plates	9862	7417
3	Tailing factor	0.97	1.08
4	Resolution	----	6.13
5	%RSD	0.31	0.71

Table 4: Optimized Chromatogram results

S. No	Name	RT	Response	USP Plate Count	USP Tailing	USP Resolution
1	**Avibactam**	2.254	3157456	9862	0.97	-
2	**Aztreonam**	3.536	1538763	7417	1.08	6.13

Table 5: Results for various validation parameters

S. No	Parameters	Avibactam	Aztreonam
1	Precision (%RSD)	0.31	0.71
2	Linearity (R^2)	0.99935	0.99966
3	% Assay	100.3	100.6
4	Method Precision (%RSD)	0.40	0.51
5	Intermediate Precision (%RSD)	0.57	0.88
6	Accuracy	99.7%	99.9%
7	LOD	0.6	0.3
8	LOQ	2.0	1.0

Table 6: Forced Degradation results for Avibactam and Aztreonam

% Drug	Avibactam		Aztreonam	
	% Assay	**% Deg**	**% Assay**	**% Deg**
Control	100	0	100	0

Acid	88.9	11.1	91.0	9.0
Alkali	84.9	15.1	88.4	11.6
Peroxide	86.6	13.4	89.6	10.4
Reduction	89.2	10.8	99.3	0.7
Thermal	95.1	4.9	98.6	1.4
Photolytic	98.6	1.4	98.8	1.2
Hydrolysis	96.4	3.6	97.1	2.9

CONCLUSION

From present research work, it is concluded that it is economical and reproducible. The method was developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of avibactam and aztreonamin API and from pharmaceutical dosage form (tablets). It is inferred that the methods were found to be simple, accurate, precise, and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Acknowledgement

Authors would like to thank to the management of MLRIP (Hyderabad, Telangana) for providing the facilities required to carry out the study.

Funding Statement

The author hereby confirms no funding was used to carry out this study.

Conflict of Interest

Author confirms no conflict of interest for the developed method.

REFERENCES

1. Sai Krishna V, Anusha Y, Navya Sri Y, Anni Selviya Z, Akiful HM, Vasudha B, Narender B. Analytical method development and validation for the determination of levetiracetam in bulk and pharmaceutical formulation by RP-HPLC. International Journal of Biology, Pharmacy and Allied Sciences. 2024; 13(2):996-1008.
2. Konda Shravan Kumar, Duddagi Suchitra, Pavan Kumar Kasu, Sukanya Mandala, Chalavadi Sudha Bhargavi, Narender Boggula. RP-HPLC method for simultaneous estimation of trifluridine and tipiracil in pure and tablet dosage form. Biochem. Cell. Arch. 2024; 24(2):2519-24.
3. Sy SK, Beaudoin ME, Zhuang L, Löblein KI, Lux C, Kissel M, Tremmel R, Frank C, Strasser S, Heuberger JA, Mulder MB. In vitro pharmacokinetics/pharmacodynamics of the combination of avibactam and aztreonam against MDR organisms. Journal of Antimicrobial Chemotherapy. 2016;71(7):1866-80.
4. Sillén H, Mitchell R, Sleigh R, Mainwaring G, Catton K, Houghton R, Glendining K. Determination of avibactam and ceftazidime in human plasma samples by LC–MS. Bioanalysis. 2015;7(12):1423-34.
5. Lodise TP, Smith NM, O'Donnell N, Eakin AE, Holden PN, Boissonneault KR, Zhou J, Tao X, Bulitta JB, Fowler Jr VG, Chambers HF. Determining the optimal dosing of a novel combination regimen of ceftazidime/avibactam with aztreonam against NDM-1-producing Enterobacteriaceae using a hollow-fibre infection model. Journal of Antimicrobial Chemotherapy. 2020;75(9):2622-32.
6. Ye Q, Ding W, Rinaldi F, Huang Y, Miller SA,Bolgar M. Structural characterization of low level degradants in aztreonam injection and an innovative approach to aid HPLC method validation. J Pharm Biomed Anal. 2016; 124: 358-64.
7. Revathi Boddu, Jithendar Reddy Mandhadi, Narender Boggula, Rajeshwar Vodeti, Suchitra Duddagi. Development of Expeditious RP-HPLC Method for Simultaneous Estimation of Bilastine and Montelukast in Fixed Dosage Combination. Journal of Applied Pharmaceutical Research. 2025; 13(6):113-9.
8. Sandeep Bolla, Narender Boggula. Stability-indicating liquid chromatographic method for the simultaneous estimation of colchicine and rosuvastatin in combination. Essential Chem. 2025; 2(1):1-11.
9. Bolla, S., Boggula, N. Quantitative determination of Nebivolol Hydrochloride and Rosuvastatin Calcium in laboratory prepared mixture by robust, high-performance liquid chromatographic method. Discov. Chem. 2025; 2:61.
10. Duddagi Suchitra, Konda Shravan Kumar, Chalavadi Sudha Bhargavi, Racha Spandana, Puli Ramya Krishna, Narender Boggula. Determination of darunavir and cobicistat simultaneously using RP-HPLC in bulk and dosage form. Biochem. Cell. Arch. 2024; 24(2):2381-9.
11. Konda Shravan Kumar, Duddagi Suchitra, Pavan Kumar Kasu, Sukanya Mandala, Chalavadi Sudha Bhargavi, Narender Boggula. RP-HPLC method for simultaneous estimation of trifluridine and tipiracil in pure and tablet dosage form. Biochem. Cell. Arch. 2024; 24(2):2519-24.
12. Boggula N, Bhadru B, More K. RP-HPLC Method Validation for Levomilnacipran Estimation in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023; 14(4):900-3.
13. Narender Boggula, Dr. P. Shanmuga Pandiyan. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage Forms. Int J Pharm Sci & Res. 2021; 12(1):314-20.
14. Narasimha Kanjarla, Bhuvanachandra Pasupuleti, Narender Boggula, Praveen K Kusuma, Daniel Kothapally, Vamshikrishna Gone, Gangarapu Kiran. A HPLC-MS/MS method for the determination of Nadolol in rat plasma: Development, validation, and

application to pharmacokinetic study. European Journal of Mass Spectrometry. 2023; 29(3):170-80.
15. Abdul Rasheed, Ramachandran D and Jameelunnisa Begum, Spectrophotometric determination of Aztreonam in Bulk and its Pharmaceutical Formulations. Asian Journal of Chemistry. 2010; 22(4): 2491-4.
16. Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.
17. Akula Sushma,Konda Shravan Kumar, Narender Boggula, Duddagi Suchitra. RP-HPLC Method Development and Validation of Lopinavir and Ritonavir in Bulk and Pharmaceutical Dosage Forms. Advances in Bioresearch. 2024; 15(5):280-9.
18. Gouthami Thumma, Narender Boggula, Vasudha B, Anand Kumar Chettupalli. Method Development and Validation for the Simultaneous Analysis of Duloxetine HCL and Methylcobalamine by RP-HPLC. International Journal of Pharmaceutical Quality Assurance. 2018; 9:363-7.