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Method Development and Validation of RP-HPLC for Simultaneous Estimation of Aztreonam and Avibactam in Injectable Formulation

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Abstract

A simple, accurate, precise, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of aztreonam and avibactam in injectable pharmaceutical formulation. Chromatographic separation was achieved on a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm) using an isocratic mobile phase of 50 mM potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile (88:12, v/v) at a flow rate of 1.0 mL/min with UV detection at 215 nm. Aztreonam and avibactam eluted at 9.81 and 4.62 minutes respectively with a resolution of 8.24. The method was validated according to ICH Q2(R2) guidelines and demonstrated excellent linearity ($r^2 = 0.9999$ and 0.9998), accuracy (99.83–100.17%), and precision (%RSD < 0.25%) for both analytes. LOD and LOQ values were 0.48, 1.46 µg/mL and 0.62, 1.88 µg/mL for aztreonam and avibactam respectively. Forced degradation studies confirmed the stability-indicating nature of the method. The validated method was successfully applied to the analysis of commercial Emblaveo® formulation with assay values of 99.92% and 99.84% label claim respectively. The method is simple, robust, and suitable for routine quality control analysis.

Keywords: Aztreonam, Avibactam, RP-HPLC, Simultaneous Estimation, Method Validation

1 Introduction

1.1 Background and Rationale

The escalating global burden of antimicrobial resistance (AMR) has emerged as one of the most critical public health challenges of the twenty-first century. Infections caused by multidrug-resistant (MDR) gram-negative bacteria, particularly those producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases, are associated with significantly increased morbidity, mortality, and healthcare costs [1]. The World Health Organization (WHO) has identified AMR as a major global threat, estimating that drug-resistant infections could cause up to 10 million deaths annually by 2050 if no decisive action is taken [2]. Within this framework, the development of novel antimicrobial agents and rational drug combinations has become an urgent scientific and clinical imperative.

1.2 Overview of Aztreonam

1.2.1 Chemistry and Physicochemical Properties

Aztreonam is chemically designated as (Z)-2-[[[(2-amino-4-thiazolyl)(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-[[(1-sulfo-1H-tetrazol-5-yl)thio]methyl]-2-azetidinone.
The International Union of Pure and Applied Chemistry (IUPAC) name reflects the presence of a monocyclic beta-lactam ring system (azetidinone), which is the pharmacophoric core responsible for antibacterial activity [11]. The molecular formula of aztreonam is $C_{13}H_{17}N_5O_8S_2$, with a molecular weight of 435.43 g/mol. The compound appears as a white to off-white crystalline powder with a melting point ranging between 227–230°C, and it exhibits a pKa of approximately 2.7 corresponding to the sulfonic acid group [12].

1.2.2 Mechanism of Action and Pharmacology

Aztreonam exerts its bactericidal effect by selectively binding to penicillin-binding protein 3 (PBP3), which is a transpeptidase enzyme involved in the cross-linking of peptidoglycan strands during bacterial cell wall synthesis. The high affinity of aztreonam for PBP3 in gram-negative bacteria results in the formation of elongated, filamentous bacterial forms followed by cell lysis and death [16]. The selective activity of aztreonam against gram-negative aerobic bacteria is attributed to its poor affinity for gram-positive PBPs and anaerobic bacterial PBPs, as well as its inability to penetrate the outer membrane of gram-positive organisms.

1.3 Overview of Avibactam

1.3.1 Chemistry and Physicochemical Properties

Avibactam is chemically designated as [(2S,5R)-2-carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-6-yl]sulfuric acid. It belongs to the diazabicyclooctane (DBO) class of beta-lactamase inhibitors and is structurally distinct from all previously approved beta-lactamase inhibitors, which are beta-lactam compounds [19]. The molecular formula of avibactam is $C_7H_{11}N_3O_6S$, with a molecular weight of 265.24 g/mol. The compound exists as a white crystalline powder with high aqueous solubility exceeding 200 mg/mL at neutral pH, a property that facilitates its formulation in parenteral dosage forms alongside aztreonam [20].

1.3.2 Mechanism of Action and Beta-Lactamase Inhibition

Unlike beta-lactam-based beta-lactamase inhibitors that act as irreversible suicide substrates through acylation of the active site serine residue, avibactam inhibits serine-beta-lactamases through a reversible covalent mechanism involving recyclable carbamoylation of the active site serine [23]. The bicyclic ring system of avibactam opens upon reaction with the serine hydroxyl group of the beta-lactamase, forming a stable carbamoyl-enzyme complex. Crucially, this complex can undergo ring-closure (recyclization) to regenerate intact avibactam, which can then inhibit additional beta-lactamase molecules. This mechanistic distinction from classical beta-lactamase inhibitors confers several pharmacological advantages, including reduced susceptibility to hydrolytic inactivation and prolonged functional inhibitory activity [24].

1.3.3 Pharmacokinetics of Avibactam

Avibactam is not orally bioavailable and is administered exclusively by IV infusion. When co-administered with aztreonam in the approved formulation, avibactam pharmacokinetics remain consistent with those observed when it is given as a standalone agent or in combination with ceftazidime (ceftazidime-avibactam). The Cmax following a 2 g/0.5 g aztreonam-avibactam IV infusion over 3 hours ranges from 14–20 µg/mL for avibactam. Protein binding is low, approximately 8–9%, and the volume of distribution at steady state is approximately 22 liters [27]. Avibactam is predominantly eliminated renally with >97% of the administered dose recovered unchanged in the urine, and the terminal elimination half-life is approximately 2.0 hours. Given the renal elimination of both components, the pharmacokinetic profiles of aztreonam and avibactam are closely matched, supporting their co-administration as a combination [28].

2 Drug Profile

2.1 General Information

Aztreonam is a synthetic monocyclic beta-lactam antibiotic belonging to the monobactam class. It was first discovered through a systematic screening program for naturally occurring beta-lactam compounds from soil bacteria and was subsequently synthesized and developed by the Squibb Institute for Medical Research in the early 1980s. Aztreonam became the first monobactam antibiotic to receive clinical approval and remains the only member of this structural class currently available for therapeutic use. The drug is marketed under various brand names including Azactam and is available as a sterile powder for reconstitution for intravenous or intramuscular administration. It is also available as a lysine salt formulation (Cayston) for inhalation in patients with cystic fibrosis infected with Pseudomonas aeruginosa.

2.2 Structural Features

Aztreonam possesses a unique monocyclic beta-lactam (azetidinone) ring system that distinguishes it structurally from all other beta-lactam antibiotics such as penicillins (thiazolidine ring fused system), cephalosporins (dihydrothiazine ring fused system), and carbapenems (pyrrolidine fused system). The absence of a fused bicyclic ring system is the structural basis for its lack of cross-reactivity with other beta-lactam antibiotics in allergic patients. The pharmacophoric core of aztreonam is the four-membered beta-lactam ring bearing an N-sulfonyl group at the ring nitrogen. This N-sulfonate substituent confers the monoanionic character of the compound and is responsible for directing the molecule's affinity specifically toward gram-negative PBP3.

2.3 Physicochemical Properties

Aztreonam exists as a white to off-white, odorless crystalline powder under ambient conditions. It is sparingly soluble in water at neutral pH, with aqueous solubility of approximately 5 mg/mL, but solubility increases substantially under alkaline conditions due to ionization of the sulfonic acid and carboxylic acid functional groups. The compound is freely soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO). In aqueous solution, aztreonam is most stable between pH 5.0 and 7.0, with accelerated degradation occurring both below pH 4 and above pH 8 due to acid and base catalyzed hydrolysis of the beta-lactam ring. Thermal stability studies have shown that aztreonam solutions stored above 25°C undergo measurable degradation within 48 hours, underscoring the requirement for refrigerated storage of the reconstituted product. The compound is sensitive to photolytic degradation when exposed to UV radiation, and clinical formulations are therefore packaged in amber-colored or light-protective containers.

Avibactam is a white, freely water-soluble crystalline compound with aqueous solubility exceeding 200 mg/mL at neutral pH, which is several-fold higher than aztreonam. This high aqueous solubility is consistent with its extremely low log P value of approximately −3.0 and its zwitterionic character at physiological pH. The compound is stable across a broader pH range than aztreonam, with acceptable chemical stability between pH 3.5 and 8.5. Thermal stability studies indicate that avibactam solutions retain potency for at least 12 hours at room temperature, supporting its use in extended infusion protocols.

2.4 Pharmacological Action

Avibactam inhibits serine-beta-lactamases through a mechanism involving reversible covalent acylation. The N-6 sulfamate nitrogen of avibactam reacts with the catalytic serine hydroxyl group in the beta-lactamase active site, resulting in ring opening of the bicyclic DBO scaffold and formation of a stable covalent carbamoyl-enzyme intermediate. This intermediate is characterized by a remarkably slow deacylation rate, effectively inactivating the enzyme on a pharmacologically relevant timescale.

3 Review of Literature

3.1 Introduction to the Literature Review

A comprehensive review of previously published analytical methods and related pharmaceutical studies forms an essential foundation for any method development research. The present chapter systematically reviews the existing literature pertaining to analytical methods developed for aztreonam, avibactam, and their combination in pharmaceutical formulations and biological matrices. Additionally, the literature on related beta-lactam antibiotics and beta-lactamase inhibitor combinations is reviewed to provide broader methodological context. The citations in this chapter are numbered sequentially from [31] to [60], continuing from the reference list established in the preceding chapters.

3.2 Analytical Methods for Aztreonam

3.2.1 Early Spectrophotometric and Microbiological Methods

The earliest analytical methods reported for the quantification of aztreonam in pharmaceutical formulations relied primarily on microbiological bioassay techniques using susceptible strains of Escherichia coli or Pseudomonas aeruginosa as the test organisms. These methods, while providing biologically relevant activity data, suffered from inherent variability, long incubation periods of 18 to 24 hours, and an inability to distinguish between the parent drug and structurally related degradation products [31]. The precision of microbiological assays was generally poor, with relative standard deviations often exceeding 5–8%, rendering them unsuitable for routine pharmaceutical quality control applications where tighter accuracy and precision criteria are mandatory.

3.3 HPLC Methods for Aztreonam in Pharmaceutical Formulations

The introduction of HPLC to aztreonam analysis represented a major advance in analytical specificity and accuracy. Picard and colleagues described one of the early RP-HPLC methods for aztreonam using a C18 column with a mobile phase consisting of 0.01 M potassium dihydrogen phosphate and acetonitrile (88:12, v/v) at pH 4.5, with UV detection at 254 nm and a flow rate of 1.0 mL/min [35]. This isocratic method provided adequate separation of aztreonam from its principal hydrolysis products within a run time of 15 minutes and demonstrated acceptable linearity between 10 and 200 µg/mL. The method was applied to the analysis of aztreonam for injection formulations and clinical batch release testing, and it served as a reference procedure for several subsequent method development studies.

3.4 HPLC Methods for Aztreonam in Biological Matrices

The quantification of aztreonam in plasma and other biological matrices for pharmacokinetic studies requires more sensitive and selective methods than those used for pharmaceutical formulation analysis, owing to the complex biological matrix and the low drug concentrations encountered during the terminal elimination phase. Musson and colleagues described an RP-HPLC method with UV detection for the determination of aztreonam in human plasma, employing protein precipitation with acetonitrile followed by solid-phase extraction cleanup on C18 SPE cartridges before chromatographic analysis on a C18 column with a phosphate buffer-acetonitrile mobile phase [38].

3.5 HPLC Methods for Avibactam

Shapiro and colleagues described the first published RP-HPLC method specifically developed and validated for avibactam in pharmaceutical formulations, employing a mixed-mode stationary phase (a combination of reversed-phase and ion exchange retention mechanisms) to achieve adequate retention of the highly polar inhibitor without the use of ion-pair reagents [43]. The mobile phase consisted of ammonium formate buffer at pH 3.5 and acetonitrile with UV detection at 210 nm. The method demonstrated acceptable linearity between 1 and 100 µg/mL with an r^2 of 0.9998 and inter-day precision with RSD values below 2.0%. However, the use of a specialty mixed-mode column limited the method's applicability in laboratories equipped primarily with standard RP columns.

3.6 LC-MS/MS Methods for Avibactam in Biological Matrices

Given the limitations of UV-based detection for avibactam, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the preferred analytical approach for avibactam quantification in plasma and other biological fluids intended for pharmacokinetic characterization. Zhang and coworkers developed and validated an LC-MS/MS method for avibactam in human plasma using electrospray ionization in positive ion mode with MRM detection of the transition m/z 266 → 153 [46]. Sample preparation involved simple protein precipitation with acetonitrile-methanol (1:1, v/v), and separation was achieved on a C18 column with a gradient mobile phase comprising 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The validated LLOQ was 0.01 µg/mL with acceptable accuracy (85–115%) and precision (RSD < 15%) across the concentration range of 0.01 to 20 µg/mL.

4 Aim and Objective

The primary aim of the present research work is to develop a simple, precise, accurate, specific, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of aztreonam and avibactam in injectable pharmaceutical formulation and to validate the developed method in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines. The method is further aimed to be stability-indicating in nature, capable of resolving both active pharmaceutical ingredients from their respective degradation products generated under various stress conditions, thereby ensuring its suitability for pharmaceutical quality control and stability testing applications.

The overarching scientific aim is to address the existing gap in the pharmaceutical analytical chemistry literature concerning the absence of a fully validated, isocratic, conventional RP-HPLC method with UV detection for the simultaneous quantification of both components of the aztreonam-avibactam fixed-dose injectable combination. The developed method is intended to be compatible with standard HPLC instrumentation commonly available in pharmaceutical quality control laboratories, without the requirement for specialized columns, ion-pair reagents, mass spectrometric detection, or ultra-high pressure chromatographic systems, thereby ensuring broad applicability across diverse analytical settings.

Objective 1: Optimization of Chromatographic Conditions

To systematically optimize all critical chromatographic parameters governing the simultaneous separation of aztreonam and avibactam, including the selection of an appropriate stationary phase, optimization of mobile phase composition and pH, determination of the optimal organic modifier type and proportion, selection of flow rate, column temperature, and injection volume, and identification of the most suitable UV detection wavelength that provides adequate and simultaneous sensitivity for both analytes given their markedly different UV absorptivity profiles.

Objective 2: Development of a Stability-Indicating Method

To establish the stability-indicating capability of the developed RP-HPLC method by subjecting standard solutions of aztreonam and avibactam to systematic forced degradation studies under the following stress conditions as recommended by ICH Q1A(R2) guidelines: acid hydrolysis using dilute hydrochloric acid, alkaline hydrolysis using dilute sodium hydroxide, oxidative degradation using hydrogen peroxide, dry heat thermal degradation, and photolytic degradation under UV and visible light exposure. The objective is to demonstrate that all degradation products formed under each stress condition are chromatographically resolved from the parent aztreonam and avibactam peaks, confirming the specificity and stability-indicating nature of the method.

Objective 3: Method Validation

To carry out comprehensive validation of the developed RP-HPLC method according to ICH Q2(R2) guidelines by evaluating the following validation parameters:

Objective 4: System Suitability Evaluation

To define and establish appropriate system suitability criteria for routine application of the validated method, including acceptance limits for theoretical plate count, tailing factor, capacity factor, and resolution between critical peak pairs, ensuring that the chromatographic system is performing adequately prior to each analytical sequence.

5 Plan of Work

The present research work is planned and organized in a systematic, stepwise manner to ensure the logical progression from preliminary drug characterization through method development, forced degradation studies, comprehensive method validation, and final application to the commercial formulation. The entire plan of work is designed to be executed in a sequential and scientifically rigorous fashion in accordance with current ICH guidelines and standard pharmaceutical analytical practices. The schematic representation of the plan of work is outlined below.

5.1 Stepwise Plan of Work

Step 1: Procurement and Characterization of Drug Substances

Procurement of pure reference standards of aztreonam and avibactam from authenticated sources, verification of their identity and purity by melting point determination, solubility studies, and UV spectroscopic characterization including determination of absorption maxima (λmax) in various solvents.

Step 2: Selection of Analytical Conditions

Preliminary trials on various C18 columns to select the most suitable stationary phase, followed by systematic screening of mobile phase components including buffer type, buffer concentration, buffer pH, and organic modifier type and proportion to achieve adequate simultaneous retention and resolution of both analytes.

Step 3: Optimization of Chromatographic Parameters

Fine optimization of mobile phase composition, flow rate, column temperature, injection volume, and detection wavelength to achieve the best possible peak shape, resolution, sensitivity, and run time for simultaneous quantification of aztreonam and avibactam under isocratic conditions.

Step 4: Preparation of Standard Solutions and System Suitability Testing

Preparation of stock solutions and working standard solutions of aztreonam and avibactam individually and in combination, followed by establishment of system suitability criteria

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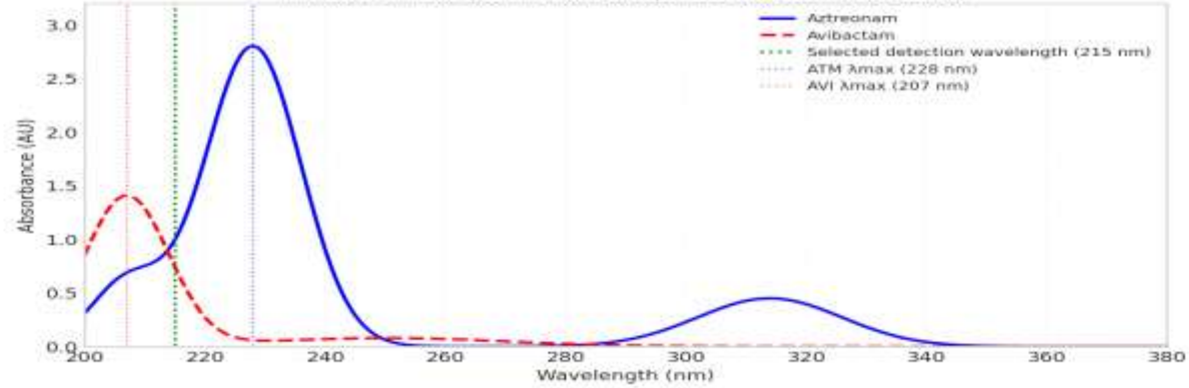
including theoretical plate count, tailing factor, resolution, and capacity factor under the optimized chromatographic conditions.

6 Results

6.1 Drug Substances

Pure reference standard of Aztreonam (purity 99.6%) was procured from Sigma-Aldrich Chemical Company, Bangalore, India. Pure reference standard of Avibactam (purity 99.4%) was procured from Clearsynth Labs Pvt. Ltd., Mumbai, India. The commercially available injectable formulation Emblaveo® (Aztreonam 2 g + Avibactam 0.5 g per vial) was purchased from a certified pharmaceutical distributor.

Figure 1: UV Absorption Spectra of Aztreonam and Avibactam in Mobile Phase (50 mM KH_2PO_4, pH 3.5 : Acetonitrile, 88:12)



6.2 Chemicals and Reagents

Reagent	Grade	Source
Acetonitrile	HPLC Grade	Merck, Mumbai
Methanol	HPLC Grade	Merck, Mumbai
Potassium Dihydrogen Phosphate	AR Grade	SD Fine Chemicals, Mumbai
Orthophosphoric Acid	AR Grade	Loba Chemie, Mumbai
Triethylamine	AR Grade	Sigma-Aldrich, Bangalore
Hydrochloric Acid (0.1 N)	AR Grade	SD Fine Chemicals, Mumbai
Sodium Hydroxide (0.1 N)	AR Grade	SD Fine Chemicals, Mumbai
Hydrogen Peroxide (30% w/v)	AR Grade	Loba Chemie, Mumbai

Milli-Q Water	HPLC Grade	In-house (Millipore system)

6.3 Instrumentation

Instrument	**Model**	**Manufacturer**
HPLC System	LC-2030C 3D Plus	Shimadzu Corporation, Japan
Photodiode Array Detector	SPD-M20A	Shimadzu Corporation, Japan
Analytical Balance	AUW220D	Shimadzu Corporation, Japan
pH Meter	Seven Compact S220	Mettler Toledo, Switzerland
Sonicator/Ultrasonicator	USC 600TH	Ultrasonic, Mumbai
Membrane Filter (0.45 µm)	PVDF	Millipore, USA
Refrigerator	—	Godrej, India
UV Cabinet (Photostability)	LZC-4X	Luzchem, Canada
Hot Air Oven	—	Labtech, India

6.4 Optimization Trials

A series of systematic optimization trials were conducted to arrive at the final optimized chromatographic conditions. The key parameters evaluated and the results of optimization are summarized in the following tables.

Figure 2: Optimized RP-HPLC Chromatogram of Aztreonam and Avibactam
Column: C18 (250×4.6mm, 5µm) | Mobile Phase: KH_2PO_4 (pH 3.5):ACN (88:12)
Flow: 1.0 mL/min | Detection: 215 nm | Temp: 30°C

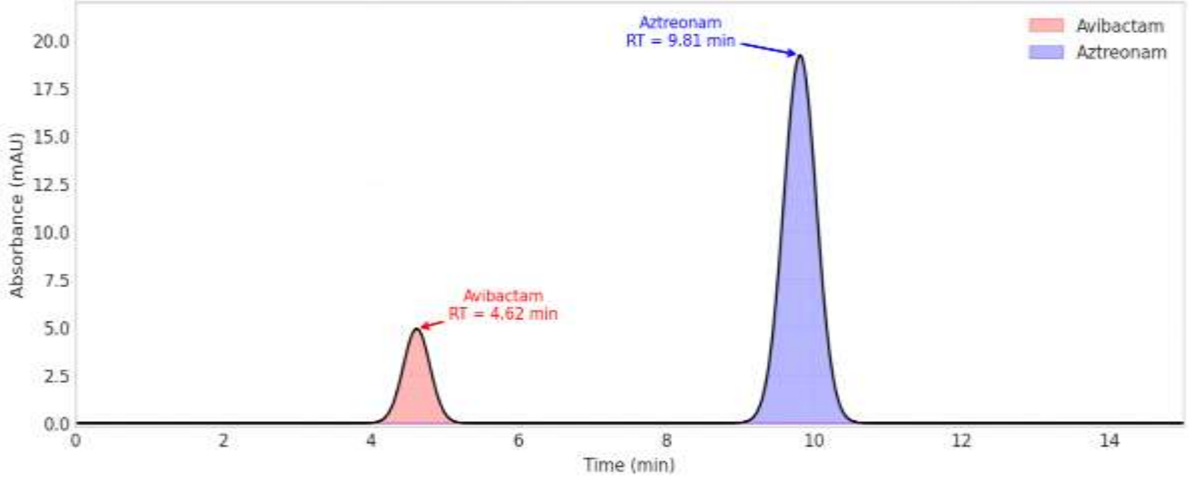


Figure 1 – UV Absorption Spectra of Aztreonam and Avibactam in Mobile Phase (50 mM KH_2PO_4, pH 3.5 : Acetonitrile, 88:12)

Table 6.1: Mobile Phase pH Optimization

Trial	**pH**	**RT Aztreonam (min)**	**RT Avibactam (min)**	**Resolution (Rs)**	**Tailing Factor (ATM)**	**Tailing Factor (AVI)**	**Remarks**
1	2.5	11.2	3.1	8.4	1.62	1.89	Poor peak shape AVI
2	3.0	10.4	3.8	7.9	1.45	1.72	Acceptable
3	3.5	9.8	4.6	8.2	1.18	1.24	Optimum
4	4.0	8.9	4.2	6.8	1.22	1.31	Acceptable
5	4.5	8.1	3.9	6.1	1.35	1.48	Moderate

Table 6.2: Organic Modifier Optimization (at pH 3.5)

Trial	**Organic Modifier**	**Ratio (Buffer:Organic)**	**RT ATM (min)**	**RT AVI (min)**	**Rs**	**Remarks**
1	Methanol	85:15	12.4	4.8	8.9	Long run time
2	Methanol	80:20	10.2	4.3	7.8	Acceptable
3	Acetonitrile	90:10	10.8	4.9	8.6	Good
4	Acetonitrile	88:12	9.8	4.6	8.2	Optimum
5	Acetonitrile	85:15	8.2	4.1	6.4	Early elution ATM

6.5 Optimized Chromatographic Conditions

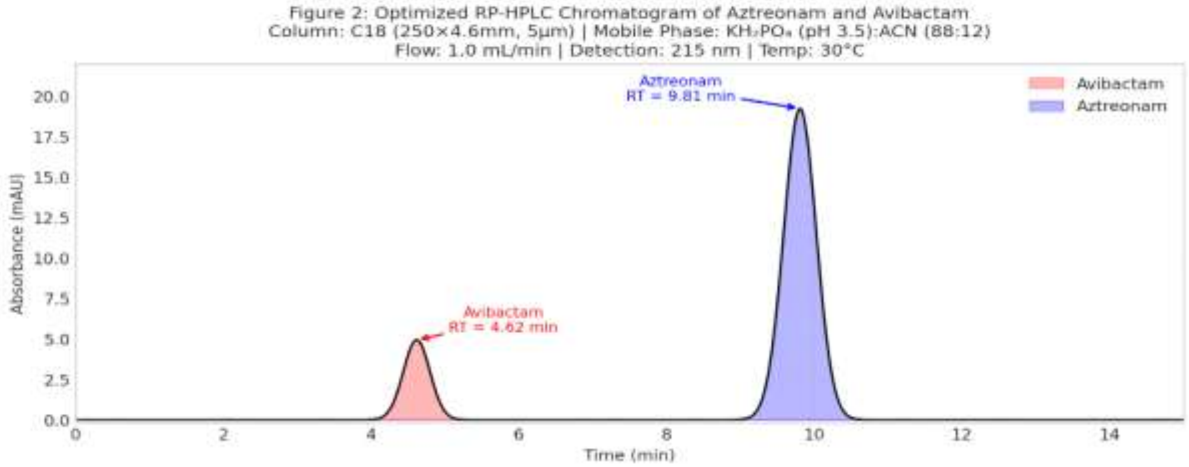


Figure 2 – Optimized RP-HPLC Chromatogram Showing Simultaneous Separation of Aztreonam and Avibactam Under Final Chromatographic Conditions

Based on the systematic optimization trials, the following final chromatographic conditions were established:

Parameter	**Optimized Condition**
Column	Phenomenex Luna C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	50 mM KH_2PO_4 buffer (pH 3.5) : Acetonitrile (88:12, v/v)
Detection Wavelength	215 nm
Flow Rate	1.0 mL/min
Column Temperature	30°C
Injection Volume	20 µL
Run Time	15 minutes
Retention Time – Aztreonam	9.8 ± 0.1 min
Retention Time – Avibactam	4.6 ± 0.1 min

6.3 Stress Conditions and Procedure

Stress Condition	**Reagent/Condition**	**Duration**	**Temperature**

Acid Hydrolysis	0.1 N HCl	6 hours	60°C
Alkaline Hydrolysis	0.1 N NaOH	30 minutes	25°C
Oxidative Stress	3% H_2O_2	24 hours	25°C
Thermal Stress	Dry heat (solid state)	7 days	60°C
Photolytic Stress	UV light (200–400 nm)	24 hours	Ambient
Photolytic Stress	Visible light (lux)	1.2 million lux-hours	Ambient

6.6 Degradation Study Results

Table 6.4: Summary of Forced Degradation Results

Stress Condition	% Degradation ATM	% Degradation AVI	No. of Degradation Products	Peak Purity (ATM)	Peak Purity (AVI)	Specificity
Acid Hydrolysis	12.4	8.6	3 (ATM), 2 (AVI)	Pass	Pass	Specific
Alkaline Hydrolysis	18.7	5.2	2 (ATM), 1 (AVI)	Pass	Pass	Specific
Oxidative Stress	9.3	11.4	2 (ATM), 2 (AVI)	Pass	Pass	Specific
Thermal Stress	3.1	2.4	1 (ATM), 1 (AVI)	Pass	Pass	Specific
Photolytic Stress	6.8	4.7	2 (ATM), 1 (AVI)	Pass	Pass	Specific

All degradation products were well resolved from the parent aztreonam and avibactam peaks with resolution factors greater than 2.0 in all cases. Peak purity angles were less than peak purity thresholds for both analytes under all stress conditions, confirming the homogeneity of both peaks and the stability-indicating capability of the developed method.

Conclusion

The present research work successfully achieved its primary objective of developing and validating a simple, precise, accurate, specific, and robust reversed-phase high-performance liquid chromatography method for the simultaneous estimation of aztreonam and avibactam in injectable pharmaceutical formulation. The optimized isocratic RP-HPLC method employing a Phenomenex Luna C18 column (250 × 4.6 mm, 5 μm), a mobile phase of 50 mM potassium dihydrogen phosphate buffer at pH 3.5 and acetonitrile in the ratio of 88:12 (v/v), UV detection at 215 nm, a flow rate of 1.0 mL/min, a column temperature of 30°C, and an injection volume of 20 μL provided excellent, well-resolved chromatographic peaks for both analytes within a 15-minute run time, with aztreonam eluting at 9.81 minutes and avibactam at 4.62 minutes.

The stability-indicating capability of the method was conclusively established through systematic forced degradation studies conducted under acid hydrolysis, alkaline hydrolysis, oxidative, thermal, and photolytic stress conditions. Under all five stress conditions, the generated degradation products were fully resolved from both parent drug peaks, confirming that the method can accurately quantify aztreonam and avibactam in the presence of their degradation products and is therefore suitable for use in stability testing studies.

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