

Measuring Aztreonam Variations by Process Analytical Technology

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RAPID COMMUNICATION

Abstract

Aztreonam for injection (Fresenius Kabi) is a sterile, nonpyrogenic, sodium-free lyophilized, off-white to slightly yellow solid containing approximately 780 mg of arginine per gram of aztreonam, indicated for the treatment of specific infections caused by susceptible Gram-negative microorganisms. Ensuring the physical and chemical homogeneity of this lyophilized matrix is critical for consistent clinical efficacy; however, near-infrared (NIR) spectrometric screening coupled with high-dimensional principal component analysis (PCA) reveals complex, multidimensional inconsistencies within the commercially available product. Initial localized screening demonstrated significant intra-lot variability, with spectral data points forming a distinct toroidal pattern characteristic of cyclical oscillatory dynamics and an unstable mathematical average state. Extreme multidimensional outliers extending up to 5.6 standard deviations from the data centroid were observed diverging along multiple orthogonal

trajectories, indicating that the intra-lot instability is driven by multiple independent physical and chemical mechanisms rather than a single processing failure. Expanding the evaluation to a comprehensive inter-lot analysis of 152 vials across 12 manufacturing lots confirmed that these inconsistencies persist at a systemic scale. The primary principal components divided the spectral library into two major, statistically distinct subpopulations, with asymmetric outlier distributions suggesting intermittent lapses in process control. Furthermore, projections in higher-order dimensions (PCs 7 through 9) isolated an entire manufacturing lot due to batch-level structural deviations possibly driven by trace moisture and aliphatic framework fluctuations. Nonparametric probability-density contour evaluations utilizing the FSOB algorithm and quantile-quantile (QQ) plots mathematically validated the presence of distinct subpopulations ($r_{tn}=0.99$, $r_{ts}=0.88$, $p=0.02$). Ultimately, these findings indicate that the manufacturing process is subjected to multiple, independent sources of instability, suggesting operations may intermittently fall outside a strict state of control.

Introduction

The University of Kentucky's (UK) Drug Quality Task Force (DQTF) was established in August of 2019 to engage in consumer-level quality assurance screening for drugs used within UK HealthCare's pharmacies ([Isaacs, 2025a](#)). The DQTF currently screens medications using Fourier transform near-infrared spectrometry (FTNIR) and Raman spectrometry for potential quality defects indicated by variability in absorbance peak intensities and locations. Through years of continuous monitoring, DQTF has assembled a spectral library containing medications typically used in a health system setting. Statistical analyses using the DQTF spectral library are performed to identify potential intra-lot and inter-lot variability in medications under review. Using Medwatch and publications in the scientific literature, the DQTF reports its findings in an effort to hold manufacturers accountable for GMP requirements and to improve patient outcomes by providing information on quality to augment the information on price that is already available. The increasing transparency is designed to improve the pharmaceutical supply chain.

Drug Product

Aztreonam for injection (Fresenius Kabi) is a sterile, nonpyrogenic, sodium-free lyophilized, off-white to slightly yellow solid containing approximately 780 mg arginine per gram of aztreonam. Following constitution, the product is for intramuscular or intravenous use. Aqueous solutions of the product have a pH in the range of 4.5 to 7.5 ([Fresenius Kabi, 2022](#)).

According to the official Fresenius Kabi prescribing information for Aztreonam for Injection, USP, the drug is indicated for the treatment of specific infections caused by susceptible Gram-negative microorganisms.

While the package insert covers all supplied strengths (500 mg, 1 g, and 2 g), the 2 g dose is specifically recommended for moderately severe systemic infections (administered every 8 or 12 hours) and severe systemic or life-threatening infections (administered every 6 or 8 hours). Aztreonam is indicated for the treatment of the following infections when caused by susceptible aerobic Gram-negative pathogens (such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* species, among others):

1. Urinary Tract Infections: Both complicated and uncomplicated infections.
2. Lower Respiratory Tract Infections: Including pneumonia and bronchitis.
3. Septicemia
4. Skin and Skin-Structure Infections: Including those associated with postoperative wounds, ulcers, and burns.
5. Intra-abdominal Infections: Including peritonitis.
6. Gynecologic Infections: Including endometritis and pelvic cellulitis.

Aztreonam is also indicated for adjunctive therapy in surgery to manage infections caused by susceptible Gram-negative organisms, including during intra-abdominal or gynecologic procedures where mixed aerobic/anaerobic infections are suspected (often requiring concurrent use of an anti-anaerobic agent).

Because aztreonam is active only against Gram-negative aerobic pathogens, the package insert strongly recommends concurrent initial therapy with other antimicrobial agents (such as those active against Gram-positive or anaerobic bacteria) in seriously ill patients who are at risk of having a mixed infection before the causative organism is known.



Figure 1. Vials of Fresenius Kabi aztreonam for injection drug product..

[Figure 1](#) is a photo of the Fresenius Kabi aztreonam drug product. A small sample of lot numbers 6037395, 6037576, 6037671 were screened initially. Six vials were screened from each lot prior to conducting the interlot analysis using the spectral library. The 12 lot numbers making up the spectral library were 6023367, 6023454, 6024160, 6031740, 6034917, 6035079, 6035564, 6035939, 6036397, 6036985, 6037458, and 6037576.

Background

Recent studies

The most significant clinical development for aztreonam over the past three years has been its formulation as a fixed-dose combination with the beta-lactamase inhibitor avibactam (ATM-AVI). This combination restores aztreonam's activity against multidrug-resistant Gram-negative bacteria that co-produce metallo- β -lactamases (MBLs) and serine β -lactamases. This research culminated in the European Medicines Agency (EMA) approval of ATM-AVI in April 2024 and the U.S. FDA approval, under the brand name Emblaveo, in February 2025 for adults with complicated intra-abdominal infections (cIAI) who have limited treatment options. The following details the pivotal clinical studies and pharmacokinetic modeling published between 2023 and 2026.

The REVISIT trial (NCT03329092)([Carmeli, 2025](#)), a Phase 3 study published in *The Lancet Infectious Diseases* in October 2024, served as the cornerstone supporting recent regulatory approvals by providing robust efficacy and safety data against established carbapenem regimens. Designed as a prospective, multinational, open-label, central assessor-masked, and randomized (2:1) trial, it evaluated 422 hospitalized adults with cIAI, hospital-acquired pneumonia (HAP), or ventilator-associated pneumonia (VAP) caused by Gram-negative bacteria. Patients received either ATM-AVI, with or without metronidazole for cIAI, or meropenem, with or without colistin, for 5 to 14 days. For cIAI, adjudicated clinical cure rates at the test-of-cure visit were 76.4% for the ATM-AVI cohort versus 74.0% for the meropenem cohort, with 28-day all-cause mortality rates of 1.9% and 2.9%, respectively. In cases of HAP/VAP, clinical cure rates were 45.9% for ATM-AVI compared to 41.7% for meropenem, while 28-day mortality was 10.8% versus 19.4%. The safety profile of ATM-AVI was consistent with aztreonam monotherapy, and no treatment-related serious adverse events were reported in the ATM-AVI group.

While the REVISIT trial captured a broad array of Gram-negative pathogens, the Phase 3 ASSEMBLE trial (NCT03580044) ([Daikos, 2025](#)) specifically targeted difficult-to-treat MBL-producing infections. Published in *JAC-Antimicrobial Resistance* in July 2025, this prospective, multicenter, randomized, open-label trial evaluated hospitalized adults with cIAI, complicated urinary tract infections (cUTI), HAP/VAP, or bloodstream infections confirmed to be caused by MBL-producing Gram-negative bacteria, predominantly *Klebsiella pneumoniae*. Patients received either ATM-AVI, with or without metronidazole, or the Best Available Therapy

(BAT) for 5 to 14 days. Among patients with confirmed MBL-producing bacterial infections, clinical cure was achieved in 41.7% of those treated with ATM-AVI, compared to 0% of those receiving BAT. Furthermore, the 28-day all-cause mortality rate was notably lower in the ATM-AVI group at 8%, compared to 33% in the BAT group.

Following the Phase 3 trials, advanced population pharmacokinetic and pharmacodynamic (PK/PD) models were finalized in 2025 to optimize dosing regimens across varying degrees of renal function. As detailed in *Antimicrobial Agents and Chemotherapy* in June 2025 ([Xie, 2025](#)), a simultaneous ATM and AVI population model confirmed the efficacy of the recommended Phase 3 dosing regimen. This regimen, consisting of a 30-minute loading dose immediately followed by a 3-hour extended loading dose, and subsequent 3-hour maintenance infusions every 6 hours, achieves a joint probability of target attainment (PTA) of >89% at steady state. The regimen effectively met critical PD targets, specifically maintaining the free aztreonam concentration above a nominal minimum inhibitory concentration (MIC) of 8 mg/L for 60% of the dosing interval ($fT > MIC \geq 60\%$) and ensuring sufficient avibactam exposures across all renal function groups, excluding end-stage renal disease, which was not trialed.

In addition to these completed trials, several ongoing and real-world studies are actively evaluating ATM-AVI between 2023 and 2026. A Phase 2a multicenter, observer-blinded study ([NCT05639647](#)) is currently assessing the pharmacokinetics, safety, and tolerability of intravenous ATM-AVI in pediatric patients ranging from 9 months to under 18 years of age with serious Gram-negative infections. This trial was initiated in April 2023 and is slated for primary completion in August 2026. Furthermore, the AWARE study ([NCT07268560](#)) is an observational multicenter trial launched in late 2025 across 30 hospitals in China. This real-world study aims to evaluate the post-approval effectiveness of ATM-AVI against MBL-producing carbapenem-resistant Enterobacteriaceae (CRE) in cIAI and HAP/VAP cases.

Recalls

Recent pharmaceuticals research and formulation studies involving aztreonam have largely focused on overcoming delivery barriers for respiratory infections and optimizing the co-formulation of the drug with novel β -lactamase inhibitors. Driven by the need to combat multidrug-resistant *Pseudomonas aeruginosa* and metallo- β -lactamase (MBL)-producing pathogens, several important advances have been published between 2023 and 2026.

Inhalable Lipid Nanoparticles and Advanced Aerosolization

A major hurdle in localized pulmonary delivery is maintaining sustained drug release while preserving the stability of the antibiotic during nebulization. A pivotal 2025 study published in *Biomaterials Advances* ([Chauhan, 2025](#)) demonstrated the continuous production of

aztreonam-loaded inhalable lipid nanoparticles using hot-melt extrusion (HME) coupled with probe sonication. The researchers formulated these nanoparticles with a 0.1% concentration of the cationic polymer polyethylenimine (PEI), which facilitated sustained drug release. The resulting optimized nanoparticles exhibited a highly uniform particle size of less than 50 nm and a drug entrapment efficiency exceeding 70%. Furthermore, in vitro aerosolization studies confirmed a mass median aerodynamic diameter (MMAD) of less than 4.3 μm , indicating optimal deposition in the respirable airways. Microbiological assays revealed that the minimum inhibitory concentration (MIC) of this nanoparticle formulation was twofold lower than that of unformulated aztreonam against *P. aeruginosa*.

Multi-Species Pharmacokinetic Modeling for Dose Optimization

Advances in the theoretical pharmaceuticals and pharmacokinetics of aztreonam have also been published recently to support combination therapies. A comprehensive multi-species physiologically based pharmacokinetic (PBPK) model was detailed in mid-2025 ([Ye, 2025](#)). This study successfully predicted aztreonam's behavior across rats, dogs, monkeys, and humans, showing high consistency with experimental data. The model specifically quantified the tissue partition coefficients, revealing a high concentration of the drug in the kidneys ($K_p = 2.0$ to 3.0). This mathematically grounds the formulation optimization and dosing intervals required for renal-targeted clearance, providing a critical theoretical framework for the development of fixed-dose intravenous therapies.

Analytical Methodologies for Co-Formulations

With the regulatory approval of fixed-dose combinations like aztreonam-avibactam (Emblaveo), recent pharmaceuticals literature has heavily emphasized quality control and analytical method development. In February 2026, researchers published a validated reverse-phase high-performance liquid chromatography (RP-HPLC) method specifically engineered for the simultaneous determination of aztreonam and avibactam in bulk and lyophilized pharmaceutical formulations ([Sreelatha, 2026](#)). Because aztreonam is a monobactam and avibactam is a non- β -lactam inhibitor, they exhibit distinct degradation pathways and solubility profiles. Establishing a rapid, stability-indicating HPLC assay ensures that manufacturers can accurately monitor the degradation kinetics and entrapment efficiencies of both active pharmaceutical ingredients during the freeze-drying process.

Shortages

Aztreonam Drug Shortages and Supply Disruptions

Aztreonam, a monobactam antibiotic frequently used for patients with beta-lactam allergies who require Gram-negative coverage, has experienced multiple supply chain disruptions over the

past two decades. A significant raw material shortage emerged in 2008, severely impacting the availability of aztreonam injections and forcing clinicians to rely on alternative, sometimes broader-spectrum or more toxic, agents ([Griffith, 2012](#)). During a 2011 survey conducted by the Infectious Diseases Society of America (IDSA) Emerging Infections Network, 31 percent of responding infectious disease physicians specifically identified aztreonam as an antimicrobial in short supply. These historical shortages were largely attributed to manufacturing delays, scarcity of raw materials, and the economic fragility of the antimicrobial market, which often relies on a limited number of suppliers.

Supply challenges for aztreonam have continued into the present decade. A retrospective observational study published in 2025 detailed a national aztreonam shortage occurring between April and August 2024, prompting hospital pharmacists to heavily intervene in prescribing practices ([Mendez, 2025](#)). During this period, pharmacists reviewed aztreonam prescriptions to rule out false penicillin allergies—successfully identifying false allergies in 78 percent of cases reviewed—and prioritized the limited supply of aztreonam for patients who genuinely had no therapeutic alternatives. This demonstrates that while aztreonam remains a critical therapy for urinary, respiratory, and systemic Gram-negative infections, its supply remains highly vulnerable to manufacturing and distribution bottlenecks.

Recalls Associated with Aztreonam

While large-scale recalls of the aztreonam pharmaceutical formulation itself are relatively rare compared to its shortages, aztreonam has been directly involved in significant medical device recalls related to antimicrobial susceptibility testing. A notable instance occurred when a Class 1 device recall was initiated on October 20, 2022, for the Thermo Scientific Sensititre Gram Negative IVD AST plates (Recall Number Z-0331-2023)([FDA, 2022](#)). The recall was triggered because an internal technical investigation confirmed that the testing plates could yield false susceptible results for the *Proteae* tribe—including *Proteus* spp., *Providencia* spp., and *Morganella morganii*—when tested against aztreonam and several other beta-lactam and carbapenem antibiotics.

This diagnostic recall posed a severe risk to patient care, as reporting a false susceptibility to aztreonam could lead to inappropriate clinical use of the drug against resistant organisms, resulting in treatment failure. The recalling firm, Remel Inc., issued an urgent medical device correction instructing users not to report any susceptibility results for these specific bacterial strains when tested with aztreonam on the affected lots. This event highlights how aztreonam-related recalls extend beyond the drug product to the diagnostic tools required for its safe and effective clinical administration.

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected from inventory as part of routine medication quality screening. A representative sample of individual vials were selected for screening and noted to be stored under the conditions required by the manufacturer in their original packaging. FTNIR spectra were collected noninvasively and nondestructively through the bottom of the vials using a Thermo Scientific Antaris II FTNIR Analyzer (Waltham, MA, USA)([Isaacs, 2025b](#)).

Smoothing

Data smoothing is a technique used to remove noise from data. This can be done by fitting a smooth curve to the data, such as a cubic spline. Cubic splines are piecewise cubic polynomials that are continuous and have continuous first and second derivatives. This makes them very smooth and resistant to noise. Cubic splines can be easily fitted to data using least squares ([Matlab, 2024](#))([Pollock, 1998](#)).

Multiplicative Scatter Correction (MSC)

Multiplicative scatter correction (MSC) is a widely used spectrometric normalization technique. Its purpose is to correct spectra in such a way that they are as close as possible to a reference spectrum, generally the mean of the data set, by changing the scale and the offset of the spectra ([Isaksson, 1988](#)).

BEST (Bootstrap Error-Adjusted Single-sample Technique)

The BEST calculates distances in multidimensional, asymmetric, nonparametric central 68% confidence intervals in spectral hyperspace (roughly equivalent to standard deviations)([Dempsey, 1996](#)). The BEST metric can be thought of as a "rubber yardstick" with a nail at the center (the mean). The stretch of the yardstick in one direction is therefore independent of the stretch in the other direction. This independence enables the BEST metric to describe odd shapes in spectral hyperspace (spectral point clusters that are not multivariate normal, such as the calibration spectra of many biological systems). BEST distances can be correlated to sample composition to produce a quantitative calibration, or simply used to identify similar regions in a spectral image. The BEST automatically detects samples and situations unlike any encountered in the original calibration, making it more accurate in chemical investigation than typical regression approaches to near-IR analysis. The BEST produces accurate distances even when the number of calibration samples is less than the number of wavelengths used in calibration, in contrast to other metrics that require matrix factorization. The BEST is much faster to calculate as well ($O(n)$ instead of the $O(n^3)$ required by matrix factorization.)

Principal Components (PCs)

Principal component analysis is the process of computing the principal components of a dataset and using them to execute a change of basis (change of coordinate system) on the data, usually employing only the first few principal components and disregarding the rest ([Jolliffe, 2016](#)). PCA is used in exploratory data analysis and in constructing predictive models. PCA is commonly utilized for dimensionality reduction by projecting each data point onto only the first few principal components to obtain lower-dimensional data while preserving as much of the original variation in the data as possible. The first principal component is the direction that maximizes the variance of the projected data. The second principal component is the direction of the largest variance orthogonal to the first principal component. Decomposition of the variance typically continues orthogonally in this manner until some residual variance criterion is met. Plots of PC scores help reveal underlying structure in data.

Subcluster Detection

In typical near-infrared multivariate statistical analyses, samples with similar spectra produce points that cluster in a certain region of spectral hyperspace. These clusters can vary significantly in shape and size due to variation in sample packings, particle-size distributions, component concentrations, and drift with time. These factors, when combined with discriminant analysis using simple distance metrics, produce a test in which a result that places a particular point inside a particular cluster does not necessarily mean that the point is actually a member of the cluster. Instead, the point may be a member of a new, slightly different cluster that overlaps the first. A new cluster can be created by factors like low-level contamination, moisture uptake, or instrumental drift. An extension added to part of the BEST, called FSOB (Fast Son of BEST) can be used to set nonparametric probability-density contours inside spectral clusters as well as outside ([Isaacs, 2023c](#))([Lodder, 1988](#)), and when multiple points begin to appear in a certain region of cluster-hyperspace the perturbation of these density contours can be detected at an assigned significance level using r values, and visualized using quantile-quantile (QQ) plots. The detection of unusual samples both within and beyond 3 SDs of the center of the training set is possible with this method. Within the ordinary 3 SD limit, however, multiple instances are needed to detect unusual samples with statistical significance.

Artificial Intelligence Tools

Artificial intelligence (AI) tools, principally used for background information, include [Gemini](#) (Google LLC) and [GPT-5](#) (OpenAI). AI can be used in a variety of ways, including to brainstorm, organize thoughts, develop arguments, and edit.

Results and Discussion

Initial analysis

Smoothed spectra graphs

[Figure 2](#) serves as the primary visual foundation for the initial phase of the study's quality assurance screening. [Figure 2](#) displays 18 spectra of aztreonam that were used during the study's initial screening process. These spectra represent the absorbance of the drug product across a range of wavenumbers, specifically from 4000 to 10000 cm^{-1} .

[Figure 2](#) is an important component of the research for several reasons: It provides the raw, smoothed spectral data that forms the basis for the "Initial Analysis" section of the paper. The figure highlights areas of highest spectral variation, which are explored in greater detail in [Figure 3](#). It demonstrates that the observed variation is due to changes in the relative heights of absorbance peaks rather than the appearance of entirely new peaks, which might otherwise indicate distinct contaminants. The variability captured in these 18 spectra is what necessitates the subsequent Principal Component Analysis (PCA) shown in [Figure 4](#). This statistical progression is used to determine if the manufacturing process is operating within a state of control. By establishing this initial cluster, the researchers were able to identify specific vials (such as vial 3 and vial 9) that were several standard deviations away from the center of the cluster.

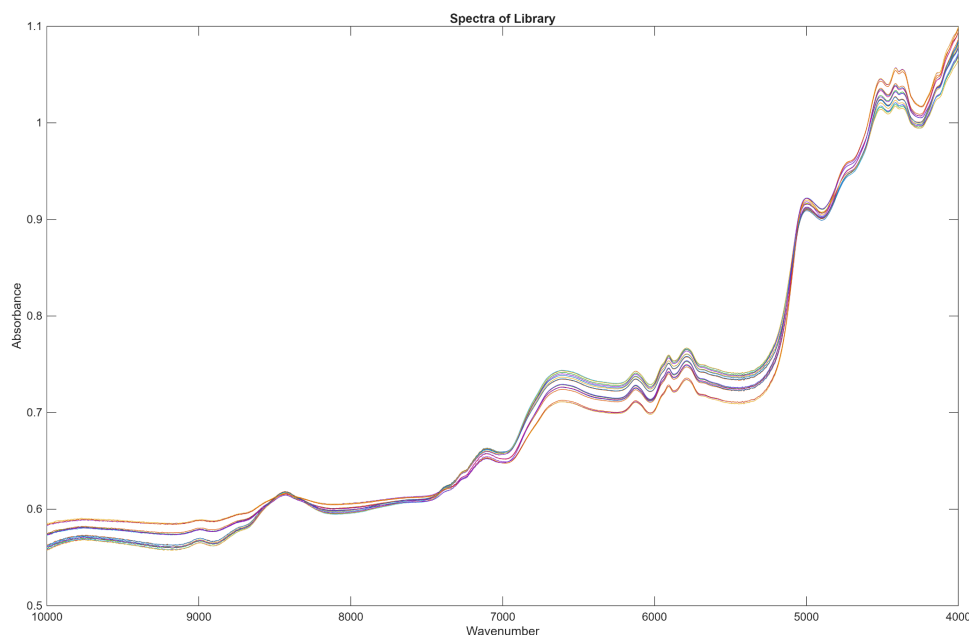


Figure 2. 18 spectra of aztreonam used in the initial screening.

[Figure 3](#) displays a magnified view of the aztreonam spectra from the initial screening, specifically highlighting one of the regions where spectral variation is at its highest. This detailed view focuses on specific absorbance peaks located at wavenumbers such as 4512, 4412, 4358, and 4999. The importance of [Figure 3](#) lies in its ability to demonstrate that the variation observed in the drug product is caused by changes in the relative heights of these peaks rather than the appearance of new, unidentified peaks. By isolating these intensities, the figure provides critical visual evidence for the potential quality defects and variability that the Drug Quality Task Force aims to detect through its spectral library analysis.

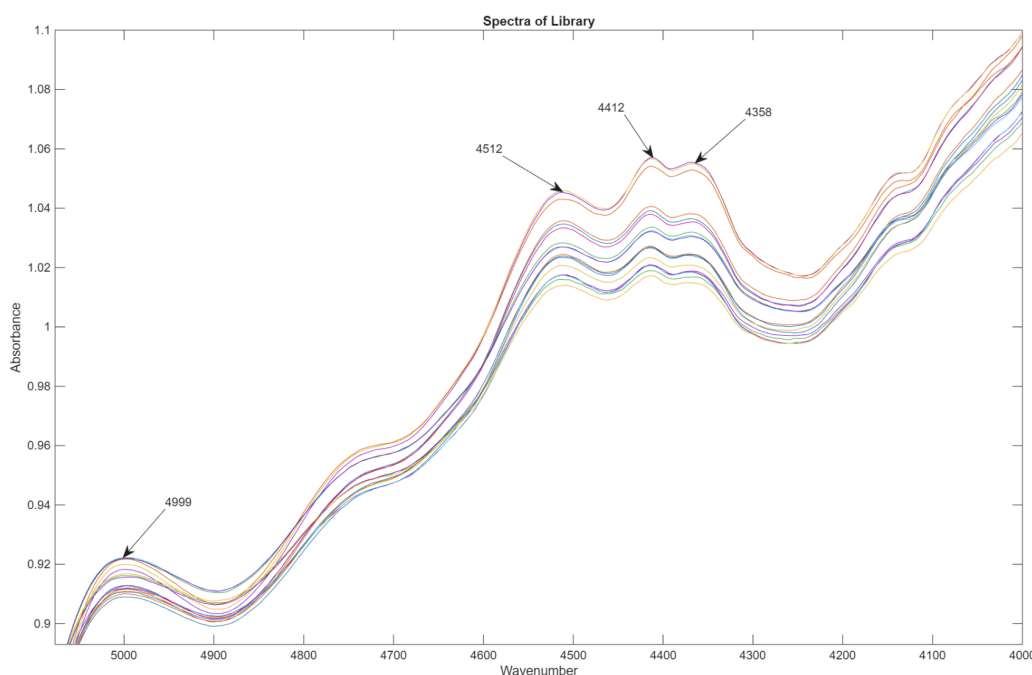


Figure 3. Zooming in on the spectra in Figure 2, one of the areas where the spectral variation is highest. The variation is due to changes in the relative heights of the peaks and not due to new peaks appearing.

PC Plots

[Figure 4](#) presents a three-dimensional scatterplot illustrating the first three principal components of the initial aztreonam spectra. Instead of forming a tight, cohesive cluster that would indicate high uniformity, the plot reveals substantial variation where the spectral data points wander around a central axis to form a distinctive toroidal, or donut-like, pattern. The importance of [Figure 4](#) stems from its role as a key diagnostic visualization of the drug's manufacturing stability. By projecting the complex spectral data into a lower-dimensional space, the figure exposes an oscillating or cyclic drift among the sampled vials. As noted in the paper's

conclusion, while this specific toroidal pattern does not definitively prove the presence of impurities or adulteration, it provides interesting evidence that the manufacturing process may have been operating outside of a stable state of process control. Ultimately, this visualization highlights the utility of principal component analysis in detecting subtle intralot variability and justifies additional investigation.

When a toroidal (donut-shaped) or ring-like pattern emerges in a Principal Component Analysis (PCA) scatterplot, it indicates a very specific geometric structure in the underlying high-dimensional data. Because PCA is a linear dimensionality reduction technique that maximizes variance, the shapes it produces are direct reflections of the dominant relationships between variables.

The primary mathematical and physical explanations for why a toroidal pattern appears in PC space are:

1. Cyclic or Oscillatory Dynamics (Limit Cycles)

This is the most common physical explanation, particularly in time-series data, continuous manufacturing, or biological systems.

- *The Mechanism:* If the underlying system is undergoing periodic oscillations (e.g., a temperature control loop "hunting" for a setpoint, seasonal variations, or a rotational mechanical process), the data points track a cyclical path in the original variable space.
- *The PCA Result:* PCA will capture this periodic variance in its primary components. Often, PC1 and PC2 will correspond to the sine and cosine components of that dominant oscillation. When plotted against each other, the orthogonal sine and cosine waves form a circle or torus. Such behavior is often observed in acoustic-resonance spectrometry ([Mills, 1993](#))([Medendorp, 2006](#))

2. The "Hollow Center" (Unstable or Theoretical Mean)

By definition, the origin point (0,0) in a PCA scores plot represents the exact mathematical mean of the entire dataset.

- *The Mechanism:* If the data forms a torus, it means the system *never actually exists* at its own statistical average.
- *The Physical Reality:* The "average" state is usually an unstable equilibrium. For example, if a chemical reactor's pressure is constantly swinging between too high and too low, the mathematical average pressure might be perfectly on target, but the physical system never actually settles there. The data are constantly pushed outward from the center, forming a ring.

3. Phase-Shifted Latent Variables

A torus often emerges when two major, independent underlying factors (latent variables) are driving the system, and they are out of phase with one another.

- *The Mechanism:* If one variable peaks while the other is at its baseline (a 90-degree phase shift), their combined variance will be captured by the first few principal components.
- *The PCA Result:* Because PCA forces the components to be orthogonal (uncorrelated) to one another, plotting these two phase-shifted variables against each other naturally yields a circular or toroidal geometry.

4. Projection of a Nonlinear Manifold

PCA is strictly a *linear* transformation. It acts like a shadow cast by a 3D object onto a 2D wall.

- *The Mechanism:* If the true underlying data lies on a complex, curved, non-linear manifold (like a 3D helix or a cylinder) in high-dimensional hyperspace, PCA struggles to unwrap it.
- *The PCA Result:* When PCA projects a 3D helix or cylinder onto a 2D plane to maximize variance, the resulting "shadow" often looks like a continuous, overlapping ring or torus. (This is a well-known limitation of PCA, which is why non-linear methods like t-SNE or UMAP are sometimes used to check if the "donut" is actually a flattened line).

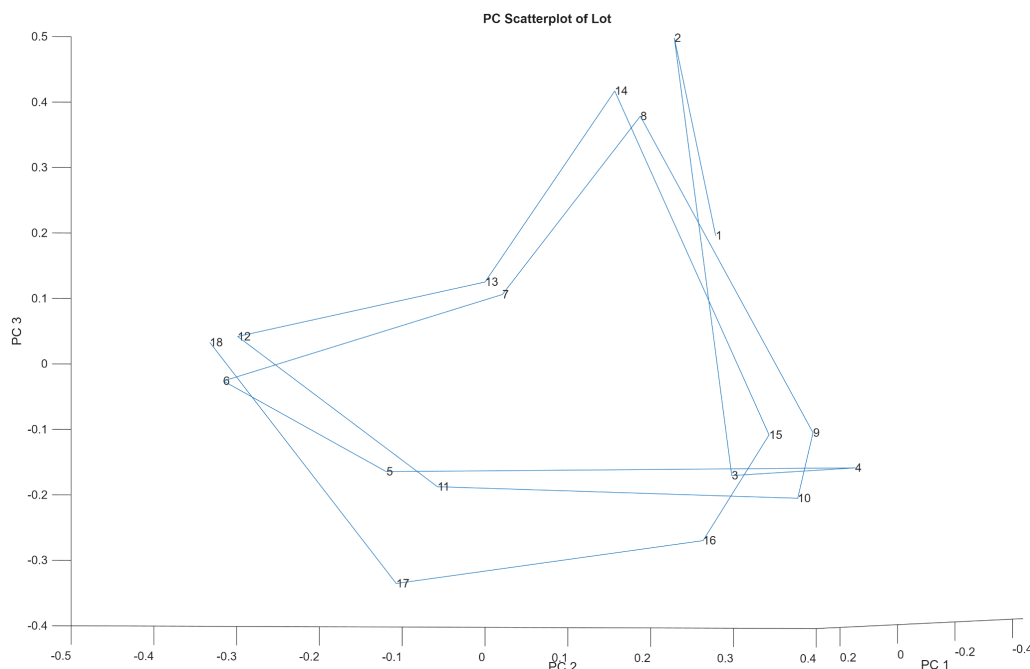


Figure 4. A plot of the first three principal components of the initial aztreonam spectra. There is considerable variation in these principal components and the spectra wander around a center forming a toroidal pattern.

[Figure 5](#) displays a three-dimensional scatterplot of the fourth, fifth, and sixth principal components (PCs 4, 5, and 6) derived from the initial aztreonam spectral data. It illustrates the spatial distribution of the sampled vials within this specific principal component space, highlighting that a couple of the vials are noticeably displaced from the main group. Specifically, the plot reveals that vial 3 is positioned 3.7 standard deviations away from the center of the cluster, and vial 9 is located even further away at 4.2 standard deviations from the center.

The importance of [Figure 5](#) lies in its ability to identify specific statistical outliers that deviate significantly from the rest of the sample. By analyzing these higher-order principal components, the researchers can pinpoint individual samples that exhibit subtle, underlying variations not fully captured by the first three principal components. This detailed outlier detection is a crucial part of identifying potential intra-lot variability, which ultimately supports the study's broader investigation into whether the drug's manufacturing process is operating outside of a strict state of control.

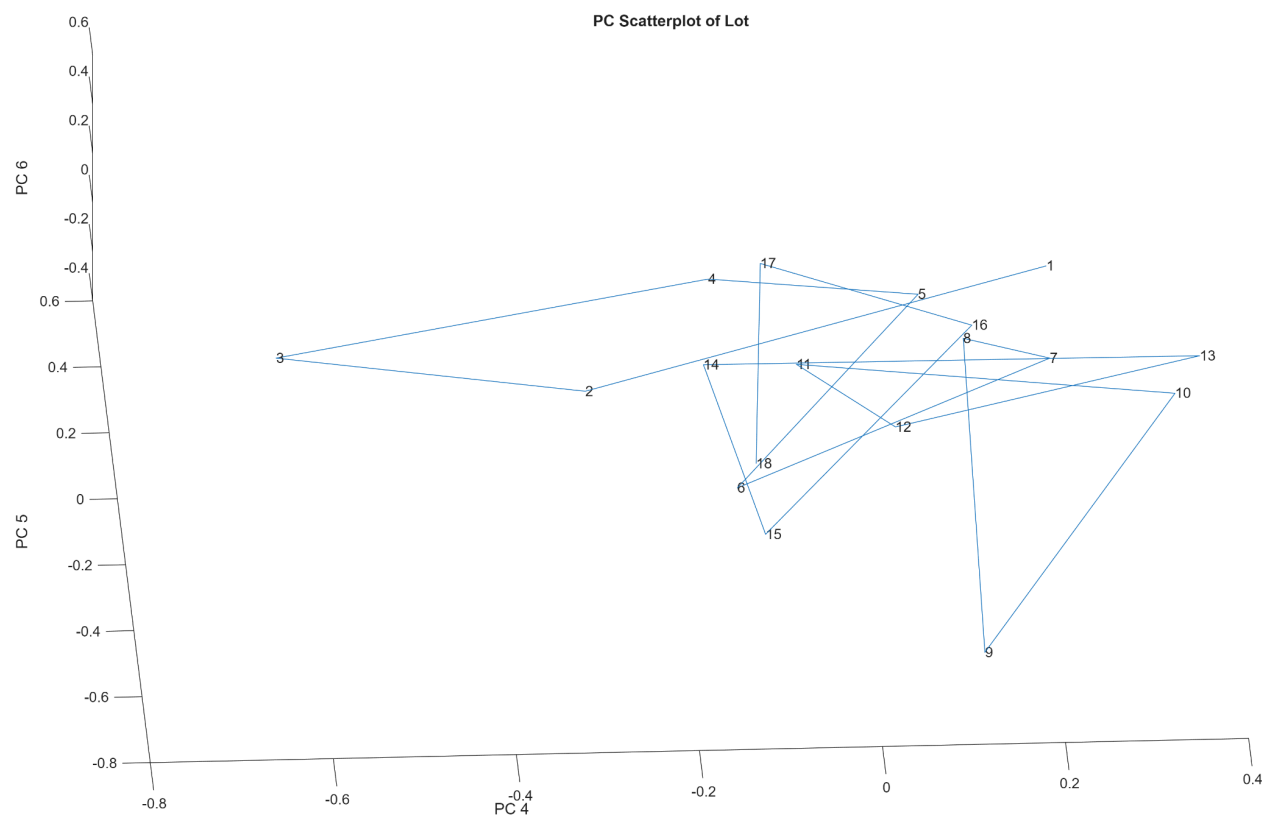


Figure 5. A plot of the next three principal components of the initial aztreonam spectra (PCs 4, 5, and 6). A couple of vials are displaced from the rest. Vial 3 is 3.7 standard deviations from the center of the cluster, while vial 9 is 4.2 standard deviations from the center.

[Figure 6](#) presents a three-dimensional scatterplot that visualizes the seventh, eighth, and ninth principal components (PCs 7, 8, and 9) derived from the initial aztreonam spectral data. Specifically, the plot identifies extreme statistical outliers within this higher-order dimensional space, most notably highlighting vial 5, which is located 5.6 standard deviations from the center, and vial 11, which sits 4.7 standard deviations away. The figure also accounts for vial 15, another outlier positioned 3.7 standard deviations from the center, though its position projects out of the visual plane of the page. The importance of this figure lies in its ability to isolate subtle, orthogonal variations in the drug's spectra that are not captured by the primary principal components. By pinpointing individual samples that deviate so drastically from the central cluster (well beyond typical statistical control limits) the figure provides critical, highly detailed evidence demonstrating significant intra-lot variability, which ultimately supports the paper's conclusion that the manufacturing process may be operating outside a state of strict process control.

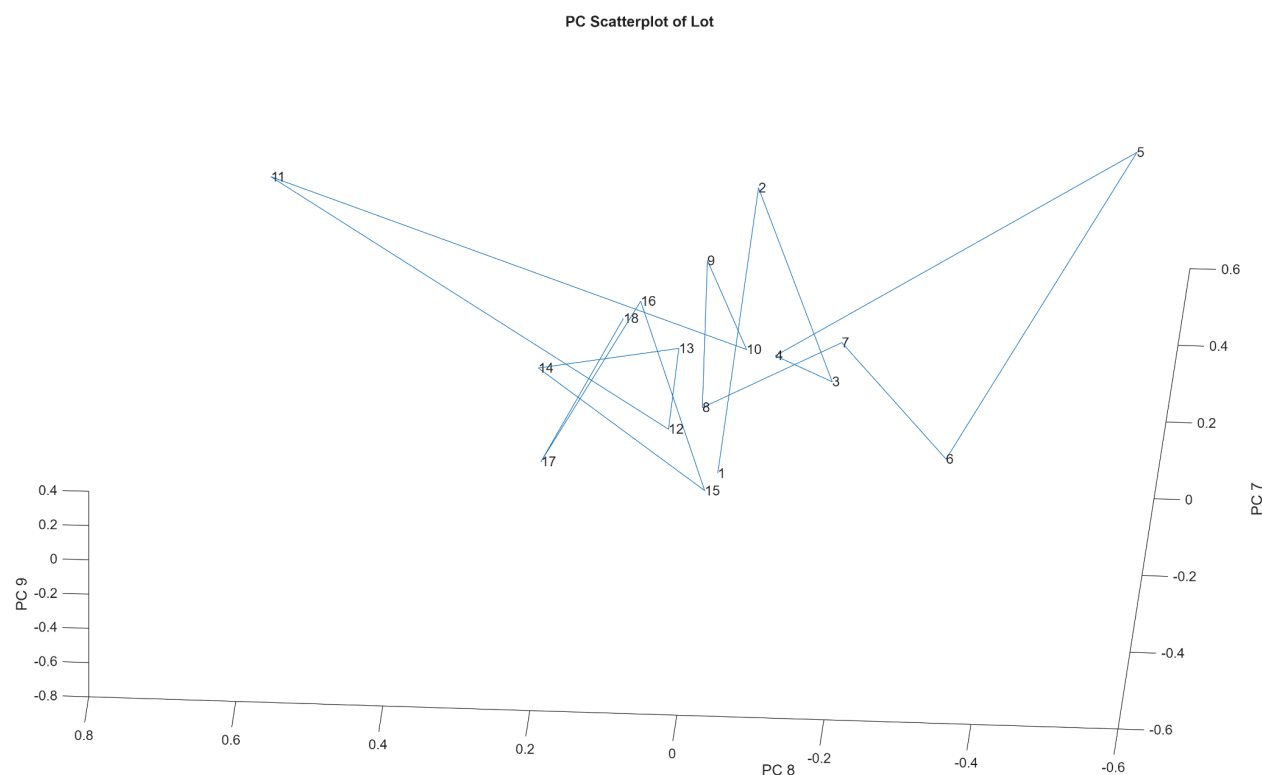


Figure 6. A plot of principal components 7, 8, and 9 shows vials 5 (5.6 SDs from the center) and 11 (4.7 SDs from the center) are outliers. Vial 15 is also an outlier (3.7 SDs) but it projects out of the page.

When a central cluster in a Principal Component Analysis (PCA) scatterplot exhibits outliers in three completely different directions, it indicates that these data points deviate from the norm for fundamentally different, independent reasons.

Because PCA axes (the principal components) represent orthogonal (uncorrelated) directions of maximum variance in the dataset, the spatial trajectory of an outlier away from the center tells a specific story about *why* it is an outlier.

The primary scientific and mathematical explanations for outliers diverging in multiple distinct directions are:

1. Distinct Physical or Chemical Mechanisms

In spectral or analytical data, moving in a specific direction in PC space corresponds to a specific change in the physical or chemical makeup of the sample (as defined by the PC loadings). If three outliers shoot off in three different vectors, each one is experiencing a unique type of deviation.

- Direction A might represent moisture contamination, pulling heavily on water absorbance bands.
- Direction B might represent a degraded active pharmaceutical ingredient or a change in polymorphism.
- Direction C might represent a purely physical irregularity, such as variations in particle size or sample packing density altering the scatter of light.

2. Independent Processing Errors

In a manufacturing context, independent failure modes project data points into different regions of the PCA plot. If a process relies on multiple independent parameters (e.g., temperature, pressure, mixing time), an outlier caused by a sudden temperature spike will look mathematically distinct (and thus spatially separate) from an outlier caused by a malfunctioning mixing blade.

3. Different Sub-Populations or Raw Material Sources

Sometimes, what appear to be isolated outliers extending in different directions are actually the leading edges of distinct, low-density sub-populations. For example, if a facility uses three different suppliers for a single raw excipient, and each supplier's material possesses a slightly different trace impurity profile, atypical samples from each of the three suppliers will pull away from the grand mean along three separate paths.

4. Independence of Variance (Orthogonality)

Because principal components are orthogonal to one another, they capture independent sources of variance.

- An outlier lying far along the positive x-axis (PC1) is driven by the variables dominating PC1.
- An outlier lying far along the positive y-axis (PC2) is driven by an entirely different, uncorrelated set of variables.
- A third outlier moving diagonally (or along a z-axis like PC3) is driven by yet another independent combination of factors.

Ultimately, the divergence of outliers in drastically different directions is a highly diagnostic feature. It confirms that the system is not simply experiencing varying degrees of a *single* problem, which would create a single stretched line or smear of outliers, but rather is being subjected to multiple, independent sources of instability or adulteration.

Table 1: Variation accounted for by each of the principal components of the spectra

PC Number	Variation in this PC	Cumulative PC Variation
1	0.8461	0.8461
2	0.1318	0.9779
3	0.0181	0.9960
4	0.0026	0.9986
5	0.0007	0.9994
6	0.0001	0.9995
7	0.0001	0.9996
8	0.0001	0.9997
9	0.0001	0.9997

[Table 1](#) displays the proportion of statistical variation accounted for by each of the first nine principal components (PCs) derived from the initial screening of the aztreonam spectral data. Specifically, the table lists both the individual fractional variance captured by each principal component and the running cumulative variance. For example, the data reveals that the first principal component alone explains approximately 84.6% (0.8461) of the total variance, while the first three components collectively account for 99.6% (0.9960) of the variance. In a typical injectable pharmaceutical, 90% or more of the total variation is usually on the first principal component. The relatively large variation in PC 1 is the first signal that this drug should be investigated more closely.

This table is important to the paper because it mathematically justifies the dimensionality reduction used throughout the initial analysis. By showing that the majority of the spectral data's

complexity is concentrated in just a few components, it validates the researchers' approach of using three-dimensional scatterplots to reliably visualize the drug's intralot variability without losing critical structural information. Furthermore, documenting the very small variance fractions in higher-order components establishes the necessary statistical context for evaluating the subtle but significant outliers detected in the corresponding plots.

Interlot analysis

Smoothed spectra graphs of library

[Figure 7](#) displays the smoothed near-infrared absorbance spectra for a comprehensive library consisting of 152 individual vials of aztreonam, which were compiled from 12 distinct manufacturing lots. The graph plots absorbance against wavenumbers ranging from 4000 to 10000, specifically highlighting structural areas of interest at wavenumbers 5007 and 4747. The importance of this figure lies in its role as the foundational visual representation for the paper's interlot analysis. While earlier figures focused on a smaller sample size to demonstrate baseline intra-lot variance, [Figure 7](#) establishes the full scope and scale of the spectral library used by the researchers. By capturing the spectral variance across a much larger and more diverse set of samples, this figure provides the necessary raw data baseline for the subsequent principal component analyses, which are used to uncover broader lot-to-lot structural groupings and systemic variations within the manufacturing process.

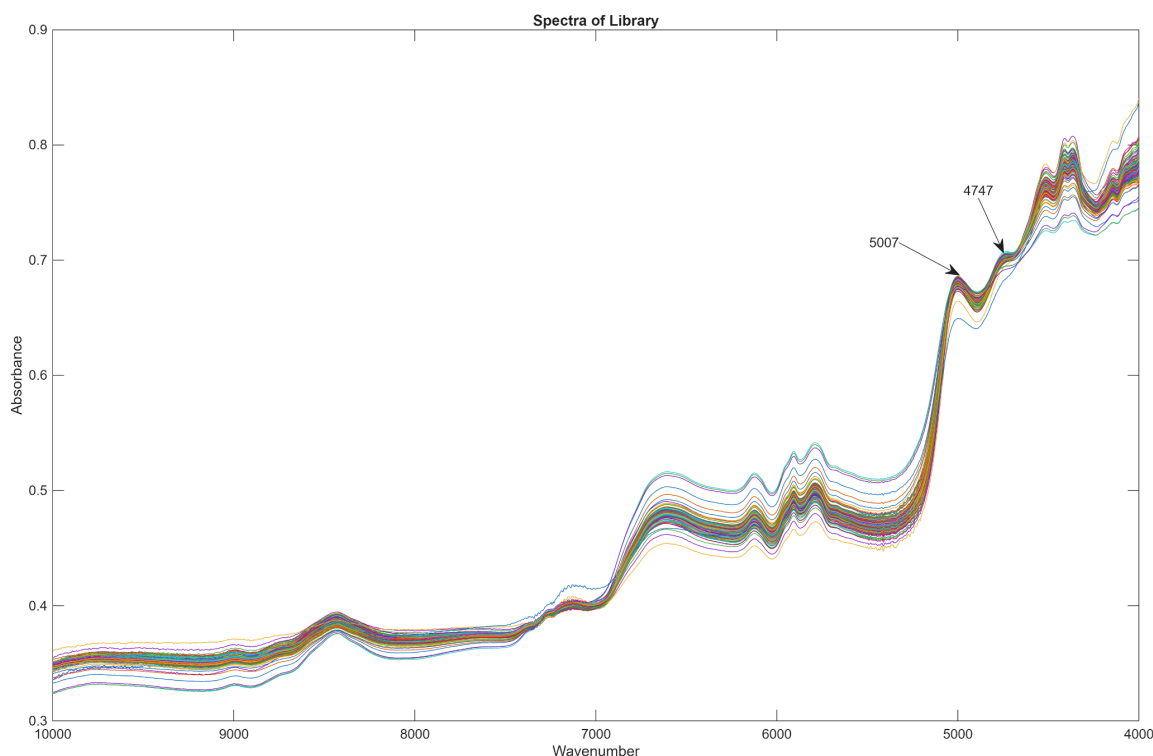


Figure 7. The spectra of all 152 vials in the aztreonam library comprising 12 different lots.

PC Plots

[Figure 8](#) presents a three-dimensional principal component scatterplot utilizing the first three principal components (PCs 1, 2, and 3) to map the spectra of the entire aztreonam library. This comprehensive library consists of 152 vials gathered across 12 different manufacturing lots. The plot visually demonstrates that the spectral data divides into two major, statistically distinct groups, noting specifically that the lower group exhibits a higher number of outliers compared to the upper group. The importance of this figure lies in its clear demonstration of interlot variability. While the earlier figures in the paper established inconsistencies within small, localized samples, [Figure 8](#) scales this analysis up to provide broad statistical evidence that the structural or chemical profiles of the drug vary significantly across different manufactured batches. The emergence of two distinct clusters rather than a single, uniform grouping strongly indicates that the manufacturing process is inconsistent over time, thereby providing crucial support for the paper's conclusion that the process may be operating outside of strict control limits.

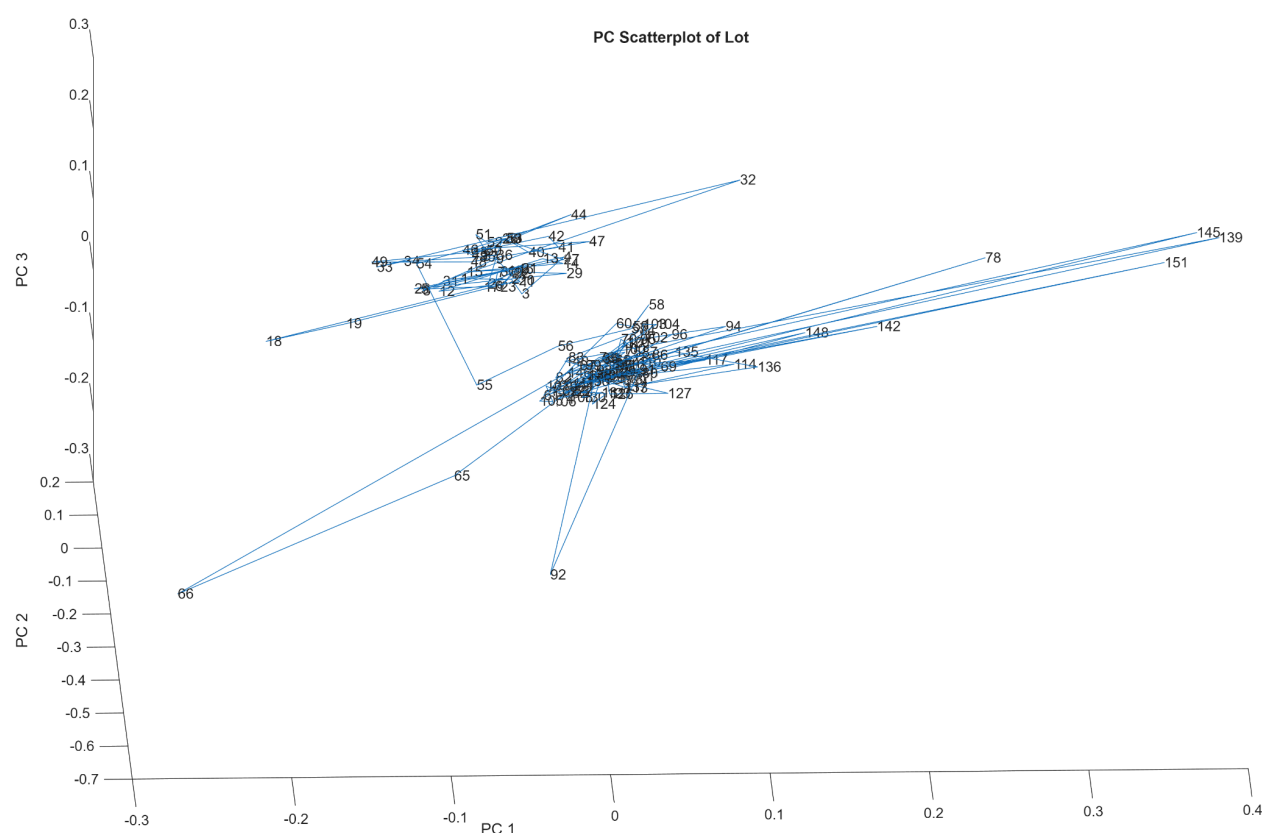


Figure 8. A plot of the spectra of the aztreonam library using PCs 1, 2, and 3. There are two major groups in the data ($r_{tn}=0.99$, $r_{ts}=0.88$, $p=0.02$), and the lower group has more outliers than the upper group.

When observing two similarly sized clusters in a principal component scatterplot where one exhibits significantly more and larger outliers, the divergence typically points to a fundamental difference in the variance and stability of the underlying data-generating processes. In pharmaceutical manufacturing and spectral analysis, a primary cluster generally represents a distinct overarching grouping of data, such as a specific production campaign, a dedicated manufacturing line, or the use of a particular raw material supplier. If one cluster is tightly grouped while the other is surrounded by extreme outliers, it provides strong statistical evidence that the latter group was subjected to a much looser state of process control or experienced intermittent, highly variable disturbances. For instance, the tight cluster might represent batches manufactured using a highly pure, homogenous active pharmaceutical ingredient under rigidly stable environmental conditions, yielding consistently uniform spectral signatures. In contrast, the cluster burdened with numerous outliers likely reflects a process fraught with sporadic inconsistencies, such as fluctuating temperature or humidity during lyophilization, intermittent equipment malfunctions, or heterogeneous mixing of excipients that only affect a fraction of the vials. Furthermore, this pattern can readily emerge from differential degradation rates or packaging discrepancies. If the outlier-heavy cluster represents an older set of lots or a run with slight variations in vial sealing integrity, non-uniform aging processes can cause a handful of samples to undergo rapid chemical degradation or moisture uptake, catapulting them into the outer regions of the principal component hyperspace while the tightly controlled lots remain uniform. Ultimately, the asymmetric distribution of outliers reveals that while the two groups share a similar baseline chemical identity to form clusters, the operational, mechanical, or environmental forces acting upon the outlier-heavy group were far less stable and reproducible.

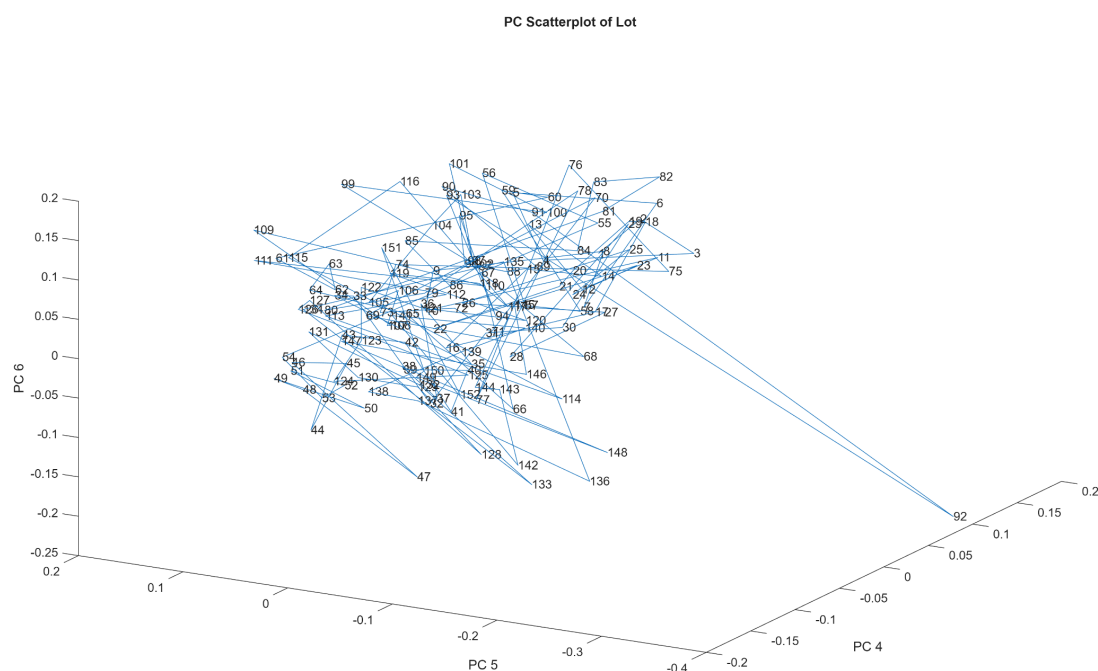


Figure 9. A scatter plot of the aztreonam library shown in [Figure 8](#), except that this figure is plotted using PCs 4, 5, and 6. The main cluster of points on the left is fairly homogeneous. One outlier, vial 92, is apparent in the plot.

[Figure 9](#) displays a three-dimensional principal component scatterplot mapping the comprehensive aztreonam spectral library, plotting the data using the fourth, fifth, and sixth principal components (PCs 4, 5, and 6). This is the same library of 152 vials from 12 lots shown in the previous figure, but projected into higher-order dimensional space. The plot reveals that the main cluster of data points on the left side is largely homogeneous, indicating general stability across these specific dimensions of variance for the bulk of the samples. However, it distinctly isolates a single prominent outlier, vial 92, which deviates substantially from the rest of the group.

The importance of [Figure 9](#) lies in its ability to uncover more subtle, orthogonal sources of spectral variation that might be overshadowed by the massive variance captured in the first three principal components. While the primary components demonstrated broad lot-to-lot inconsistencies, exploring PCs 4, 5, and 6 allows the researchers to detect unique, low-level anomalies or localized defects in individual vials. When added together, principal components 4, 5, and 6 account for 0.0324, or 3.24%, of the total spectral variation in the library. Identifying a specific outlier like vial 92 in these higher dimensions proves that even within a broadly homogeneous subset, individual units can experience independent physical or chemical deviations, reinforcing the value of comprehensive spectral screening for quality assurance.

[Figure 10](#) displays a three-dimensional principal component scatterplot mapping the inter-lot spectral data of all 152 aztreonam vials, specifically utilizing the seventh, eighth, and ninth principal components (PCs 7, 8, and 9). Unlike the earlier principal component plots that highlighted isolated, extreme individual vials, this specific visualization reveals no apparent single outliers. Instead, it demonstrates that an entire specific lot of the drug separates to form a distinct, different group from the rest of the data points. The importance of [Figure 10](#) lies in its ability to uncover systemic, batch-level deviations within the higher-order dimensions of spectral variance. While primary components capture the most obvious structural variances across the library, examining PCs 7, 8, and 9 allows the researchers to detect subtle but uniform shifts that affect a whole manufacturing run simultaneously. This finding is critical to the paper because it supports the conclusion that the manufacturing process experiences significant inconsistencies over time, resulting in entire lots that possess unique spectral signatures deviating from the standard production profile.

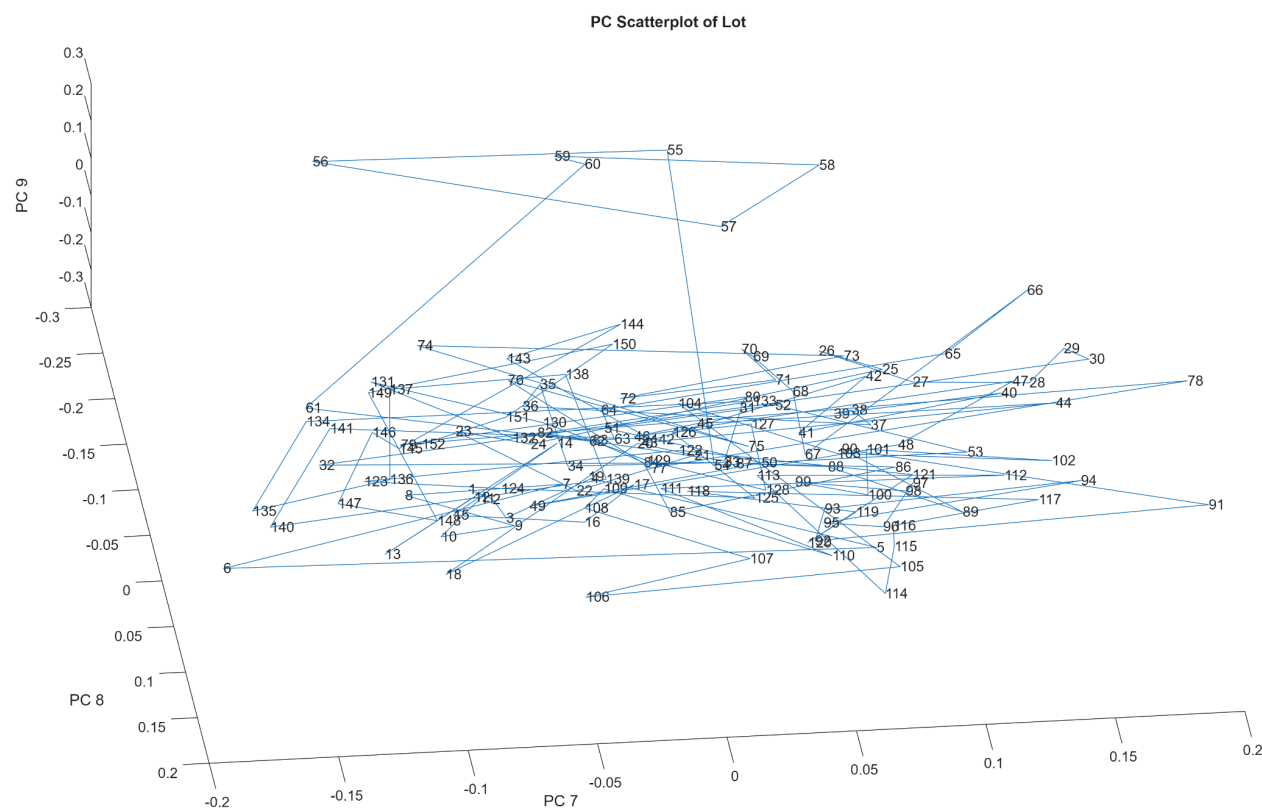


Figure 10. The plot of the same interlot data of 152 vials from [Figure 8](#) using PCs 7, 8, and 9. There are no apparent single outliers, but one lot of drug seems to form a different group.

[Figure 11](#) displays a quantile-quantile (QQ) plot of the groups within the aztreonam spectral library, plotting the Empirical Cumulative Distribution Function against the Theoretical Cumulative Distribution Function. According to the paper's methodology, this plot is generated using the Fast Son of BEST (FSOB) algorithm to visualize perturbations in nonparametric probability-density contours. These perturbations occur when multiple data points begin to aggregate in specific, anomalous regions of the spectral cluster-hyperspace. The distinct, sharp step-like shape of the plotted curve indicates a highly significant departure from a single, uniform multivariate distribution, mathematically confirming the presence of distinct subpopulations within the library.

[Figure 11](#) provides a rigorous, objective statistical foundation for the clustering visually observed in the principal component scatterplots. While the 3D plots previously demonstrated that the 152 vials from 12 different lots appeared to split into distinct physical groups, the QQ plot allows these density perturbations to be formally detected at an assigned significance level using r values. By verifying that these separate clusters represent statistically significant deviations rather than random analytical noise, the figure strengthens the paper's overarching conclusion that the drug's manufacturing process suffers from substantive lot-to-lot inconsistencies and may be operating outside of a stable state of process control.

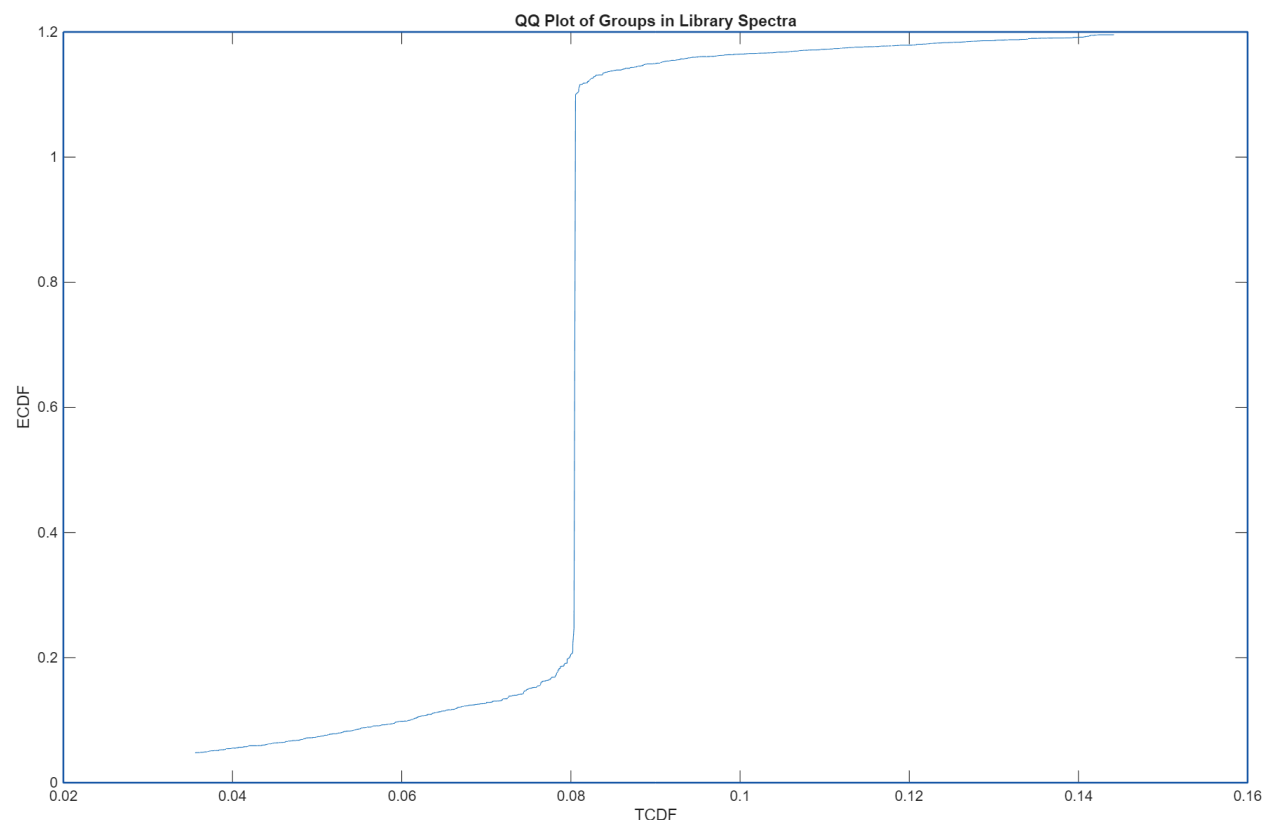


Figure 11. A QQ plot of the results of the FSOB subcluster detection algorithm on the data. There are two major groups in the data ($r_{in}=0.99$, $r_{is}=0.88$, $p=0.02$)

[Table 2](#) displays the proportion of statistical variation accounted for by each of the first nine principal components (PCs) derived from the comprehensive inter-lot spectral library of aztreonam. Specifically, the table lists the fractional variance captured by each individual principal component alongside the cumulative variance for the larger dataset, which consists of 152 vials across 12 manufacturing lots. For instance, it details that the first principal component explains approximately 80.5% (0.8054) of the total variance, while the first three components together capture roughly 96.6% (0.9658) of the spectral variation. PC 1 accounts for less than 90% of the total spectral variation, providing an early indication that process control is less than the typical injectable drug product.

The importance of [Table 2](#) lies in its role as the mathematical foundation for the dimensionality reduction utilized during the comprehensive inter-lot analysis. By proving that the overwhelming majority of the spectral library's variance is concentrated in the first three principal components, it validates the researchers' methodology of using three-dimensional scatterplots (such as [Figure 8](#)) to accurately visualize major lot-to-lot groupings without losing critical structural information. Furthermore, explicitly documenting the increasingly minute variance fractions in the higher-order components (PCs 4 through 9) provides the strict statistical context necessary

for evaluating the subtle, multidimensional outliers and batch-level shifts shown in subsequent plots ([Figure 9](#) and [Figure 10](#)). This rigorous quantification ultimately supports the study's conclusion that the observed spectral deviations represent real, mathematically significant process variations that warrant additional investigation into the drug's manufacturing control state.

Table 2: Variation accounted for by each of the principal components of the spectra in the library

PC Number	Variation in this PC	Cumulative PC Variation
1	0.8054	0.8054
2	0.1294	0.9347
3	0.0310	0.9658
4	0.0246	0.9904
5	0.0062	0.9965
6	0.0016	0.9981
7	0.0006	0.9987
8	0.0004	0.9991
9	0.0001	0.9993

PC Loadings Plots

[Figure 12](#) displays the principal component 1 (PC 1) loadings plot for the comprehensive aztreonam spectral library. The graph plots the mathematical weighting on the y-axis against the spectral wavenumbers ranging from 4000 to 10000 on the x-axis, specifically highlighting significant peaks at wavenumbers 7244 and 4885. This figure is important to the paper because it bridges the gap between abstract statistical variance and the physical chemistry of the drug product. Because Table 2 established that PC 1 accounts for the vast majority—over 80.5%—of the total variance in the inter-lot library, the PC 1 loading plot reveals the exact spectral features responsible for the largest lot-to-lot differences. By pinpointing the specific wavenumbers that have the heaviest influence on this primary component, the researchers can identify the specific molecular bonds or physical attributes of the aztreonam formulations that are fluctuating the most, providing deeper insight into why the manufacturing process may be demonstrating systemic instability.

[Figure 13](#) illustrates the loadings spectrum for the second principal component (PC 2) derived from the comprehensive aztreonam spectral library. The plot specifically highlights notable spectral peaks at the 4791, 4996, and 7044 cm^{-1} . [Figure 13](#)'s importance is firmly established by the broader discussion regarding principal component loadings plots and the interlot variance model. According to [Table 2](#), this second principal component accounts for 0.1294 of the total variation observed across the 152 vials and 12 manufacturing lots. Loadings plots are vital to this analysis because they bridge the gap between abstract statistical variance and the actual physical chemistry of the drug product. By pinpointing the specific wavenumbers that exert the

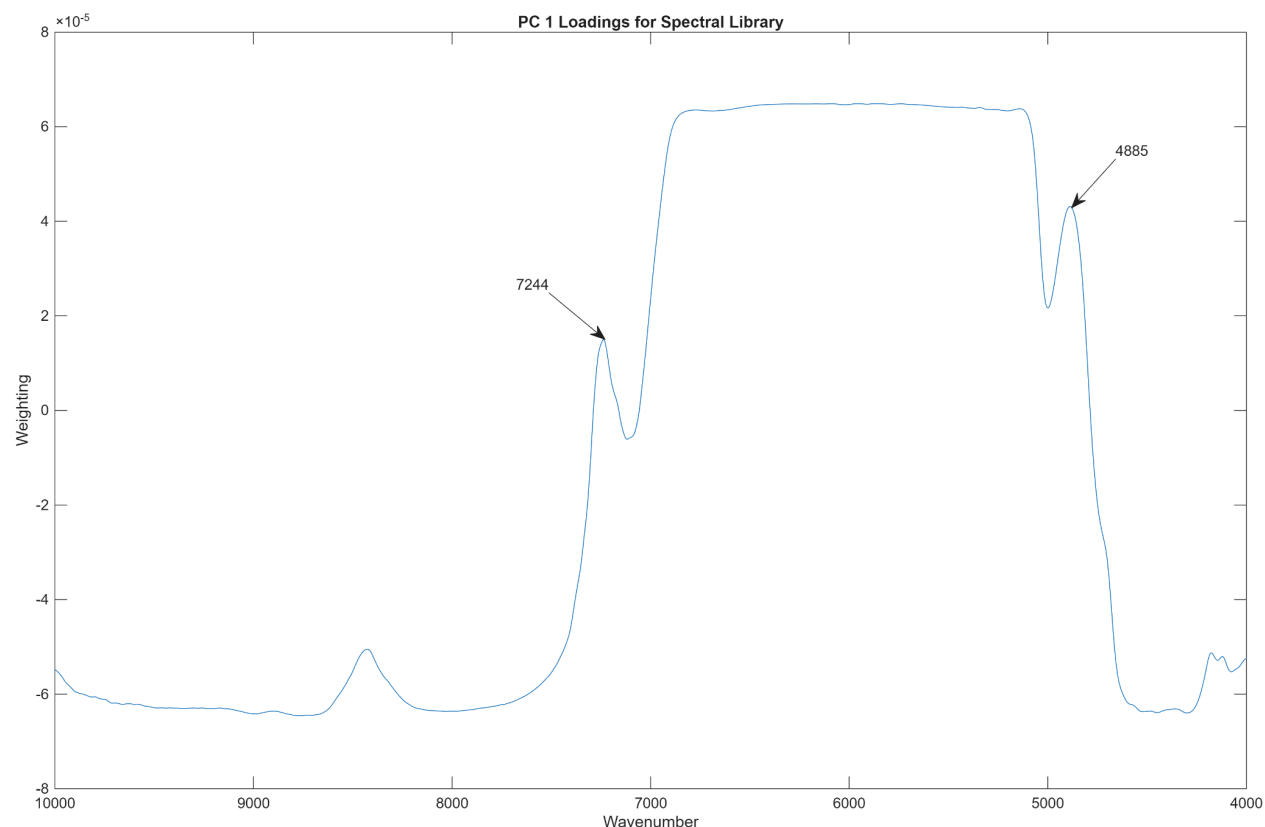


Figure 12. The loadings spectrum of PC 1 of the spectral library shows the effects of baseline correction. Notable peaks are marked at 4885 and 7244 cm^{-1} .

heaviest influence on a given principal component, researchers are able to identify the exact molecular bonds or physical attributes of the aztreonam formulations that are fluctuating the most. Consequently, [Figure 13](#) is important because it maps the specific chemical and physical features driving the secondary source of lot-to-lot variance, offering deeper insight into the exact nature of the systemic instability within the drug's manufacturing process.

In [Figure 13](#), the notable peaks occur at 4791, 4996, and 7044 cm^{-1} . These peaks correlate to specific functional groups in the drug product:

7044 cm^{-1} : This peak falls exactly within the 7090–7040 cm^{-1} range. This specific band assignment corresponds to the first overtone of O-H stretching, characteristic of free (non-hydrogen-bonded) hydroxyl groups or phenols.

4996 cm^{-1} : This wavenumber is positioned right at the boundary between the "First Overtones and O-H Combinations" region (6250 – 5000 cm^{-1}) and the "Combination Region" (5000 – 4000 cm^{-1}). It is located very near the 5128 – 5050 cm^{-1} range, which is assigned to the second overtone of the C=O (carbonyl) stretch. This carbonyl stretch overtone is often very weak and can sometimes be obscured by water.

4791 cm^{-1} : This peak lies well within the 5000 – 4000 cm^{-1} Combination Region. It sits just outside the listed 4878 – 4805 cm^{-1} band, which is assigned to the N-H (Protein / Amide) combination, specifically representing a combination of N-H stretching and Amide II bending.

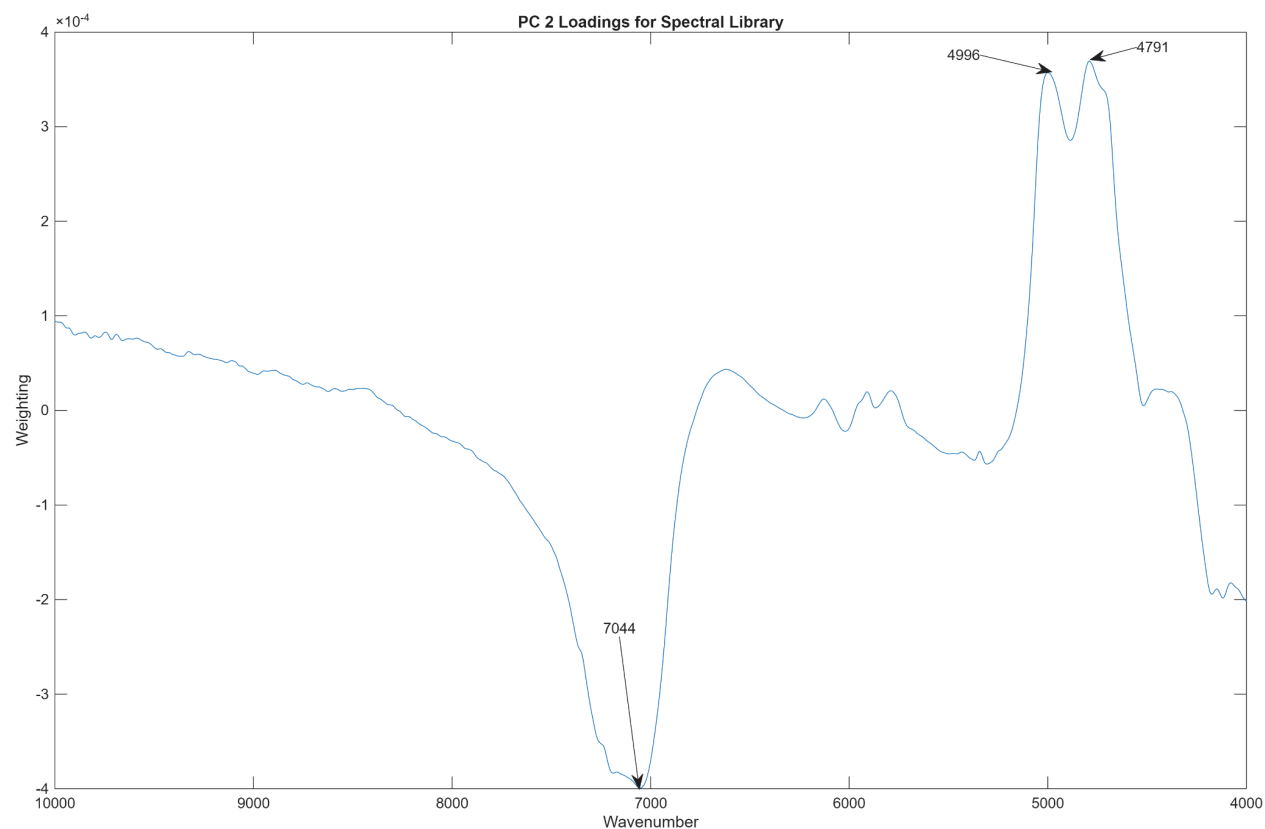


Figure 13. The loadings spectrum for PC 2 of the spectral library. Notable peaks are marked at 4791, 4996, and 7044 cm^{-1} .

Because the aztreonam molecule contains amide bonds, a sulfonic acid group, and carbonyls, these spectral assignments correlate well with the functional groups expected to be present and fluctuating in the chemical matrix.

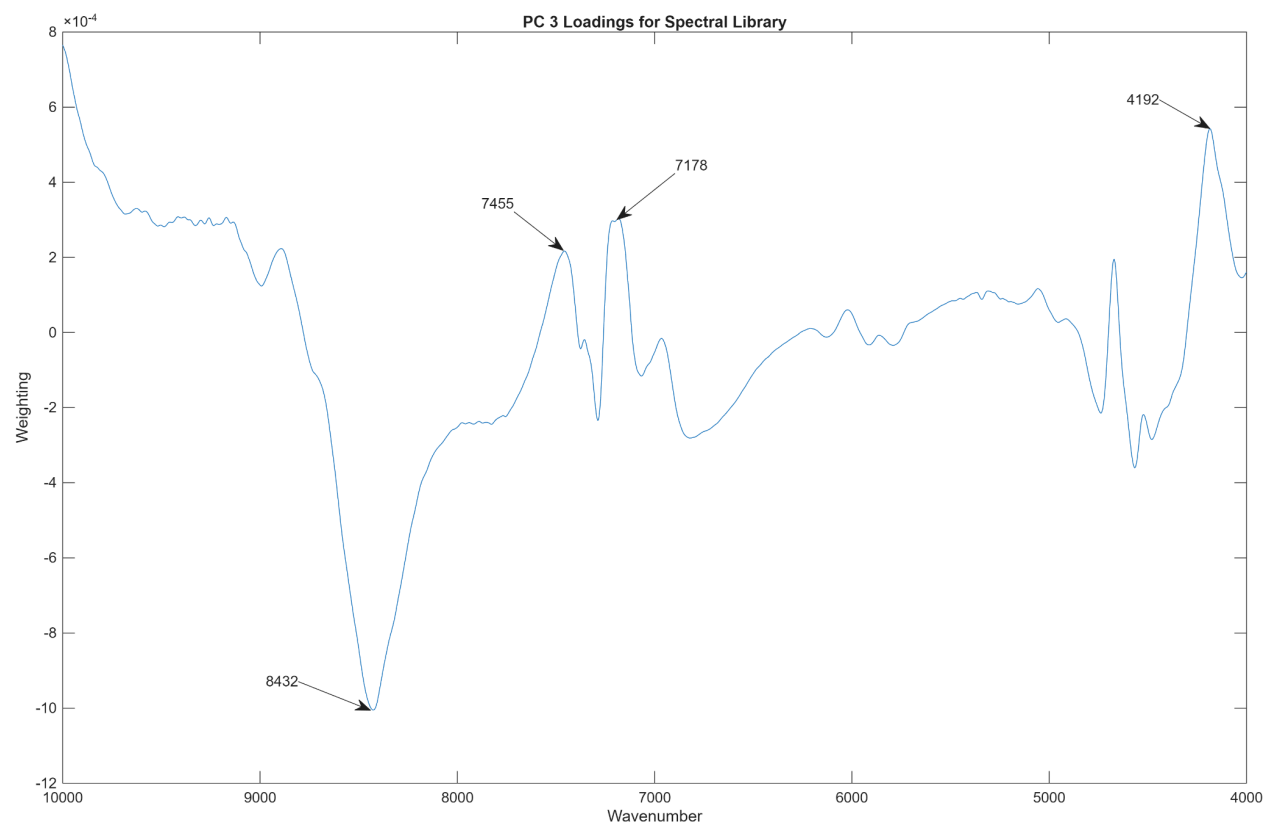


Figure 14. The loadings spectrum for PC 3 of the spectral library. Notable peaks are marked at 4192, 7178, 7455, and 8432 cm^{-1} .

[Figure 14](#) displays the loadings spectrum for the third principal component (PC 3) derived from the comprehensive aztreonam spectral library. The graph plots the mathematical weighting against the spectral wavenumbers, specifically highlighting notable peaks at 4192, 7178, 7455, and 8432 cm^{-1} . While the paper does not isolate [Figure 14](#)'s importance is established by the broader explanation of principal component loadings plots. According to the variance breakdown in [Table 2](#), PC 3 accounts for roughly 3.1% (0.0310) of the total spectral variation observed across the 152 vials and 12 manufacturing lots. Loadings plots are critical to the research because they bridge the gap between abstract statistical variance and the actual physical chemistry of the drug product. By pinpointing the specific wavenumbers that heavily influence this third principal component, researchers can identify the exact molecular bonds or physical characteristics of the aztreonam formulations that are fluctuating. Consequently, [Figure 14](#) is important because it physically maps the features driving this specific dimension of lot-to-lot variance, providing deeper insight into why the manufacturing process is exhibiting systemic instability.

Based on the PC 3 loadings spectrum shown in [Figure 14](#), the notable spectral peaks are located at 4192, 7178, 7455, and 8432 cm^{-1} . Cross-referencing these specific wavenumbers with standard near-infrared spectra-structure correlation tables provides insight into the

functional groups driving this specific dimension of variance. The highest wavenumber peak at 8432 cm^{-1} lies within the second overtone region, which is classically associated with the second overtone of C-H stretching vibrations typically found in methyl or methylene groups. The absorption bands at 7455 cm^{-1} and 7178 cm^{-1} are situated in the first overtone region; the 7455 cm^{-1} peak generally corresponds to the first overtone of C-H stretching, while the 7178 cm^{-1} peak often represents the first overtone of N-H stretching or potentially a complex combination of C-H vibrations. Finally, the peak at 4192 cm^{-1} is located deep within the combination region and is generally assigned to combinations of C-H stretching and deformation modes. Because the molecular structure of aztreonam possesses multiple aliphatic C-H bonds as well as complex amine and amide functional groups, these spectral assignments indicate that subtle physical or chemical fluctuations in the hydrocarbon framework and N-H structural environments are likely the primary factors contributing to the interlot variance modeled by this third principal component.

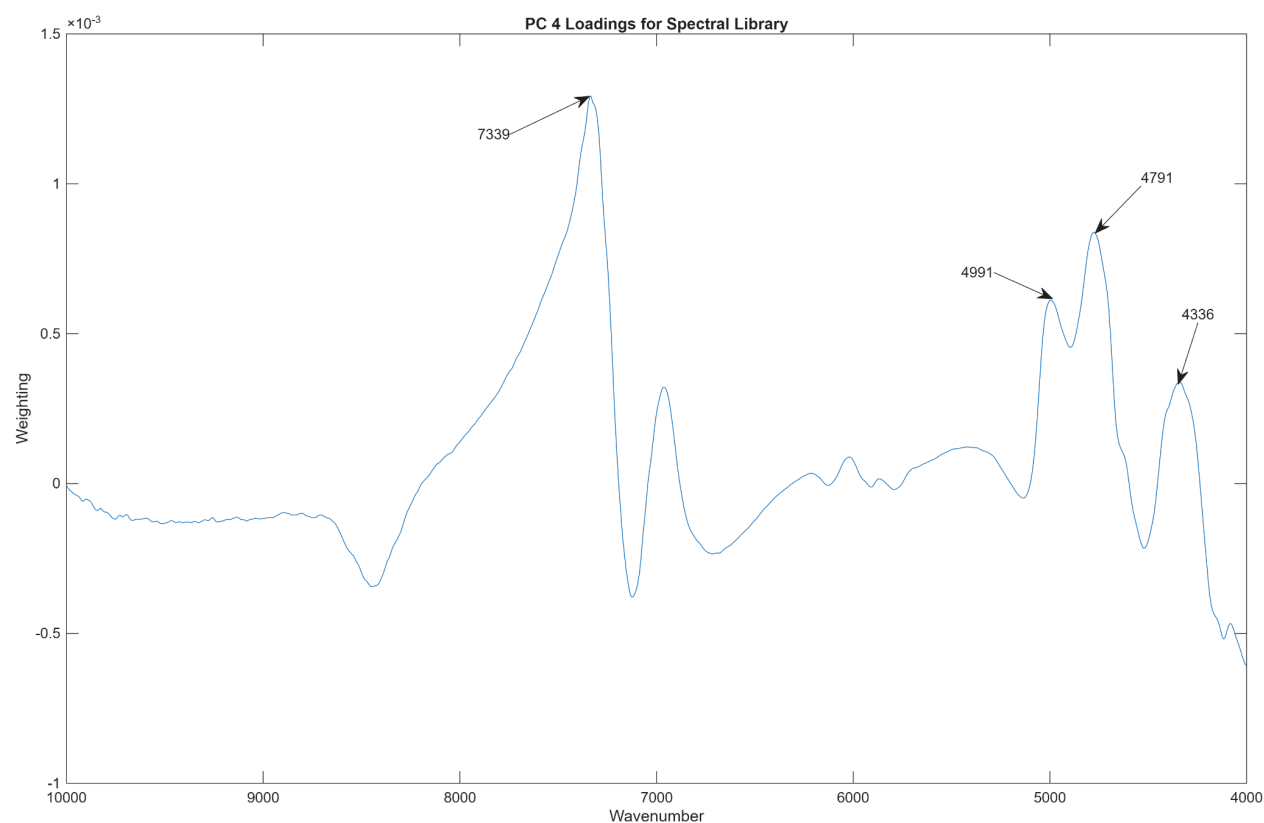


Figure 15. The loadings spectrum for PC 4 of the spectral library. Notable peaks are marked at 4336, 4791, 4991, and 7339 cm^{-1} .

Based on the loadings spectrum for the fourth principal component (PC 4) shown in [Figure 15](#), the notable spectral peaks occur at 4336, 4791, 4991, and 7339 cm^{-1} . Cross-referencing these wavenumbers with standard near-infrared spectra-structure correlations reveals the specific molecular features likely driving this dimension of variance. The peak at 7339 cm^{-1} is positioned

in the first overtone region and is characteristically associated with the first overtone of C-H stretching vibrations, which would originate from the aliphatic portions of the aztreonam molecule. The absorption at 4991 cm^{-1} sits precisely at the boundary between the first overtone and combination regions; similar to the 4996 cm^{-1} peak observed in PC 2, it is often assigned to the second overtone of a carbonyl (C=O) stretch, a signal that can be weak and sometimes obscured by environmental moisture. The 4791 cm^{-1} peak lies squarely within the combination region and represents an N-H (amide) combination band, specifically integrating N-H stretching and Amide II bending modes. Finally, the peak at 4336 cm^{-1} is classically attributed to combinations of C-H stretching and deformation (bending) modes from the hydrocarbon backbone.

[Figure 15](#) is a necessary inclusion in the report because it mathematically and physically characterizes the subtle, orthogonal sources of lot-to-lot variance that emerge after the dominant trends are accounted for. According to the inter-lot variance analysis in [Table 2](#), PC 4 captures an additional 2.46% (0.0246) of the total spectral variation within the library. While the first three principal components outline the broad structural inconsistencies across the manufacturing batches, explicitly documenting these higher-order components provides the strict statistical context necessary for evaluating multidimensional outliers. By mapping the exact wavenumbers that influence PC 4, this figure allows investigators to detect minute, localized chemical anomalies, such as independent fluctuations in the amide linkages or aliphatic framework, that independently contribute to the systemic instability of the manufacturing process.

The loadings spectrum for the fifth principal component (PC 5) shown in [Figure 16](#) identifies notable spectral peaks at 4663 , 5196 , 7233 , and 8427 cm^{-1} . Cross-referencing these wavenumbers with standard near-infrared spectra-structure correlations identifies the molecular features driving this specific dimension of variance. The peak at 8427 cm^{-1} is located within the second overtone region and is characteristically associated with the second overtone of C-H stretching vibrations from aliphatic groups. The absorption at 7233 cm^{-1} falls in the first overtone region, generally corresponding to the first overtone of C-H stretching modes. The prominent peak at 5196 cm^{-1} is typically assigned to O-H combination bands, which often strongly indicate the presence of moisture or water content, a critical factor to monitor in lyophilized pharmaceutical formulations like aztreonam. Finally, the peak at 4663 cm^{-1} lies within the combination region and is frequently attributed to combinations of C-H or N-H stretching and deformation modes. Because aztreonam contains aliphatic C-H bonds, complex amine and amide functional groups, and is susceptible to moisture uptake during manufacturing, these spectral assignments suggest that minor fluctuations in the hydrocarbon framework and trace moisture variations are contributing to the variance modeled by this component.

[Figure 16](#) is a necessary inclusion in the report because it mathematically and physically characterizes the subtle, orthogonal sources of lot-to-lot variance that emerge after the dominant trends are accounted for. According to the comprehensive interlot variance analysis in

[Table 2](#), PC 5 captures an additional 0.62% (0.0062) of the total spectral variation within the library. While the first three principal components outline the broad structural inconsistencies across the manufacturing batches, explicitly documenting these higher-order components provides the strict statistical context necessary for evaluating multidimensional outliers. By mapping the exact wavenumbers that have the heaviest influence on PC 5, this figure allows investigators to detect minute, localized chemical anomalies (such as independent moisture fluctuations or aliphatic variations) that independently contribute to the systemic instability of the manufacturing process.

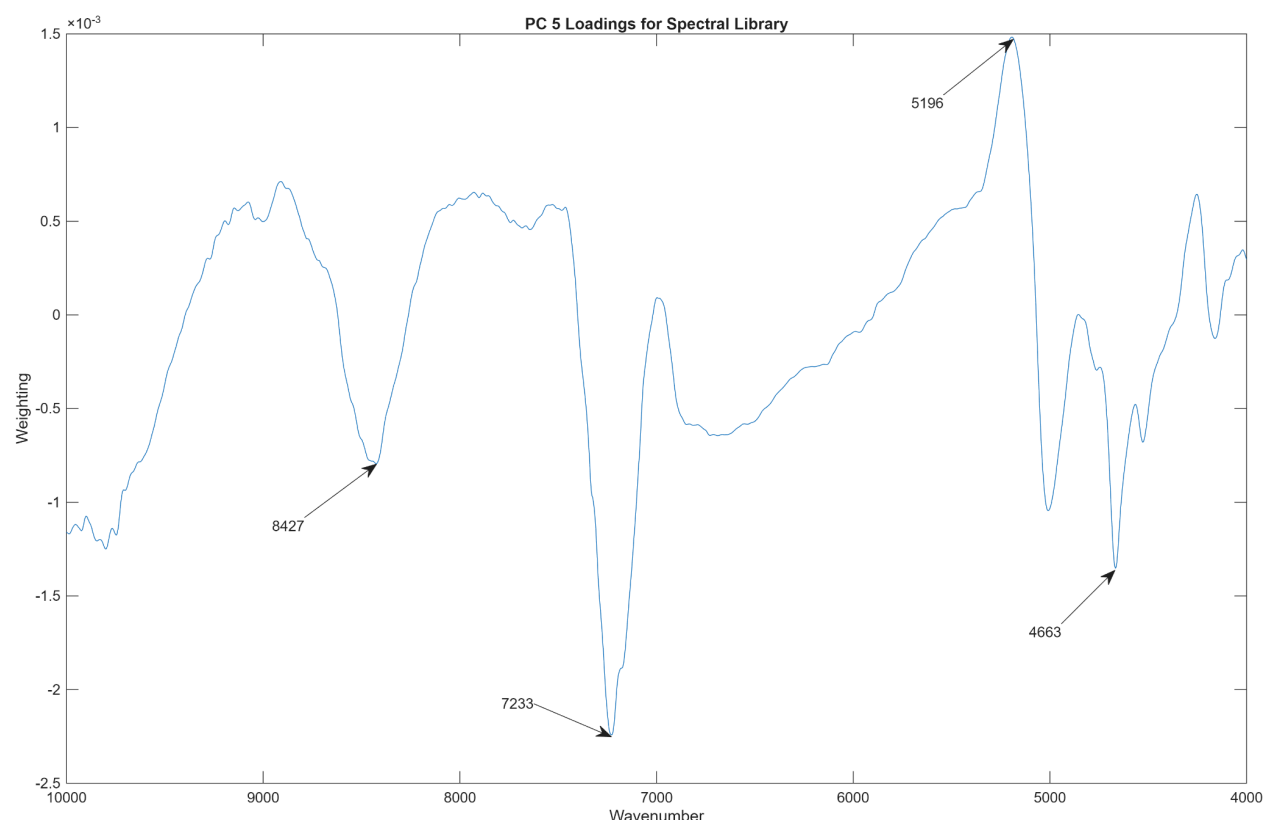


Figure 16. The loadings spectrum for PC 5 of the spectral library. Notable peaks are marked at 4663, 5196, 7233, 8427 cm^{-1} .

Based on the loadings spectrum for the sixth principal component (PC 6) displayed in [Figure 17](#), the notable spectral peaks occur at 4364, 4674, 4858, 5069, and 7106 cm^{-1} . Analyzing these wavenumbers through the lens of standard near-infrared spectra-structure correlations reveals the specific functional groups driving this highly targeted dimension of variance. The peak at 7106 cm^{-1} is located in the first overtone region and is typically associated with the first overtone of O-H or N-H stretching vibrations, suggesting fluctuations in trace moisture or the drug's amine and amide environments. The prominent absorption at 5069 cm^{-1} strongly correlates with the O-H stretch-deformation combination band, acting as a highly sensitive marker for water content

within the lyophilized matrix, though it also borders the C=O second overtone region. The peak at 4858 cm^{-1} sits squarely within the combination region and is classically assigned to N-H combinations, specifically coupling N-H stretching with Amide II bending modes inherent to the monobactam and side-chain structures of aztreonam. Finally, the absorptions at 4674 cm^{-1} and 4364 cm^{-1} represent complex combinations of C-H stretching and deformation modes originating from the aliphatic hydrocarbon backbone of the molecule.

[Figure 17](#) is an essential inclusion in this report because it mathematically isolates and physically characterizes the subtle, orthogonal sources of inter-lot variance that emerge deep within the high-dimensional hyperspace. According to the inter-lot variance analysis detailed in [Table 2](#), PC 6 accounts for just 0.16% (0.0016) of the total spectral variation within the library. While the primary components capture massive, broad structural or batch-level inconsistencies, explicitly documenting a higher-order component like PC 6 provides the rigorous statistical context required to evaluate multidimensional outliers. By mapping the exact wavenumbers driving PC 6, this figure allows investigators to detect minute, localized chemical anomalies, such as independent, low-level fluctuations in trace moisture or specific amide linkage stabilities, demonstrating that the manufacturing process is subjected to multiple, independent sources of physical and chemical instability rather than a single, uniform mode of failure.

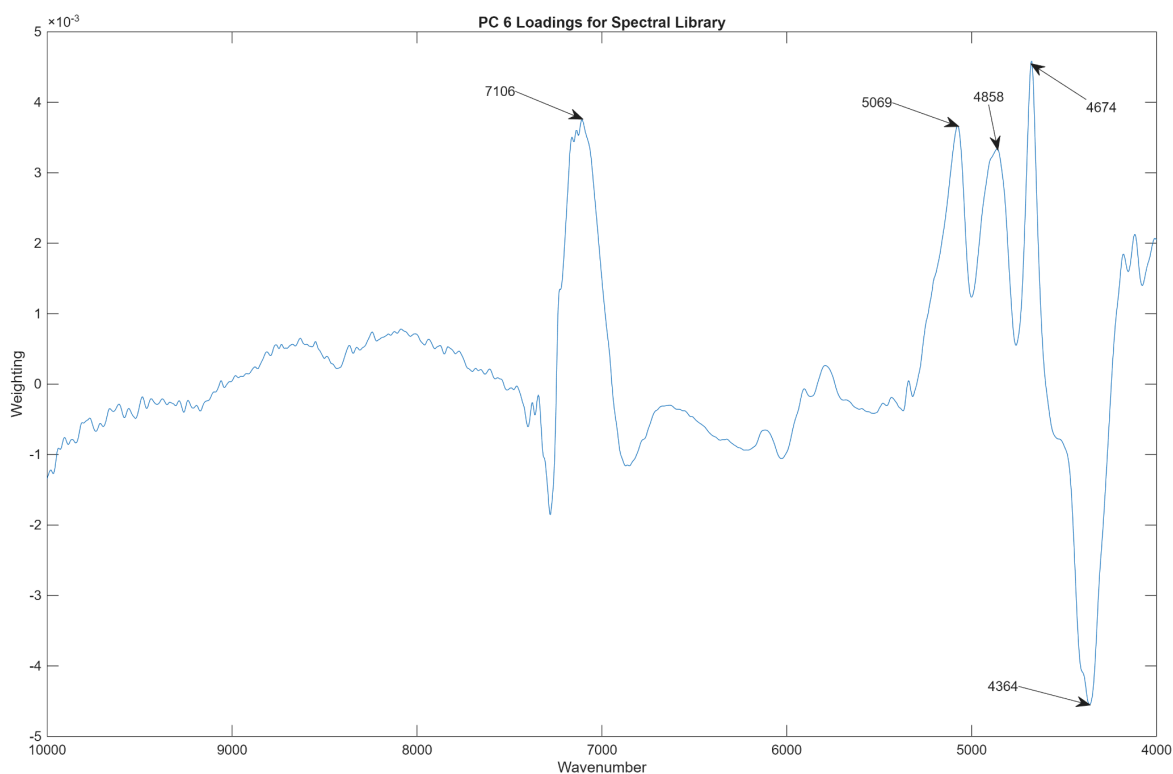


Figure 17. The loadings spectrum for PC 6 of the spectral library. Notable peaks are marked at 4364, 4674, 4858, 5069, and 7106 cm^{-1} .

Analyzing the PC 7 loadings spectrum presented in [Figure 18](#) reveals notable spectral peaks located at 4364, 4752, 5013, 5185, and 6994 cm^{-1} . Cross-referencing these specific wavenumbers with standard near-infrared spectra-structure correlations identifies the functional groups driving this specific dimension of variance. The highest wavenumber peak at 6994 cm^{-1} falls within the first overtone region and is classically associated with the first overtone of an O-H or N-H stretching vibration, pointing to fluctuations in trace moisture or the drug's complex amine and amide environments. The prominent peak at 5185 cm^{-1} is widely recognized as an O-H combination band that couples stretching and deformation modes, serving as a highly sensitive indicator for variations in hydration or trace water content within the lyophilized formulation. The absorption at 5013 cm^{-1} lies near the boundary of the combination region and is frequently assigned to the second overtone of a carbonyl (C=O) stretch, though it can also overlap with complex N-H combinations. The peak at 4752 cm^{-1} is situated well within the combination region and typically represents an N-H combination mode, specifically integrating N-H stretching with Amide II bending inherent to the aztreonam molecular structure. Lastly, the peak at 4364 cm^{-1} is characteristic of combinations of C-H stretching and deformation modes originating from the aliphatic hydrocarbon framework.

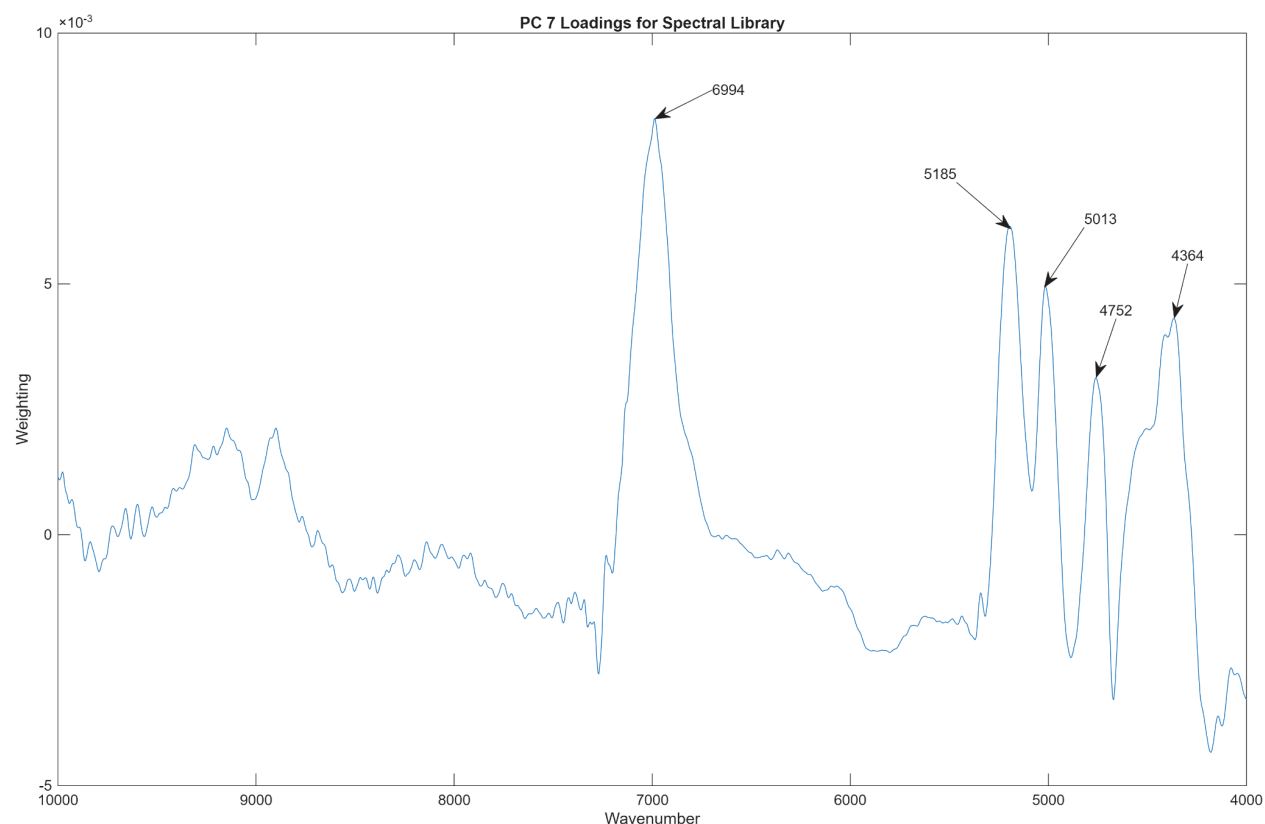


Figure 18. The loadings spectrum for PC 7 of the spectral library. Notable peaks are marked at 4364, 4752, 5013, 5185, 6994 cm^{-1} .

[Figure 18](#) is an essential inclusion in the report because it mathematically isolates and physically characterizes an extremely subtle, orthogonal source of inter-lot variance that emerges deep within the high-dimensional spectral hyperspace. According to the inter-lot variance analysis detailed in [Table 2](#), PC 7 accounts for a mere 0.06% (0.0006) of the total spectral variation across the comprehensive library. While the primary principal components capture broad, batch-level structural inconsistencies, explicitly documenting a higher-order component like PC 7 provides the rigorous statistical baseline necessary to evaluate minor multidimensional outliers and subtle probability-density perturbations. By mapping the exact wavenumbers driving this specific component, the figure allows investigators to detect minute, highly localized chemical anomalies (such as independent, low-level fluctuations in trace moisture or specific amide bond stabilities) demonstrating that the manufacturing process experiences multiple, independent sources of physical and chemical instability.

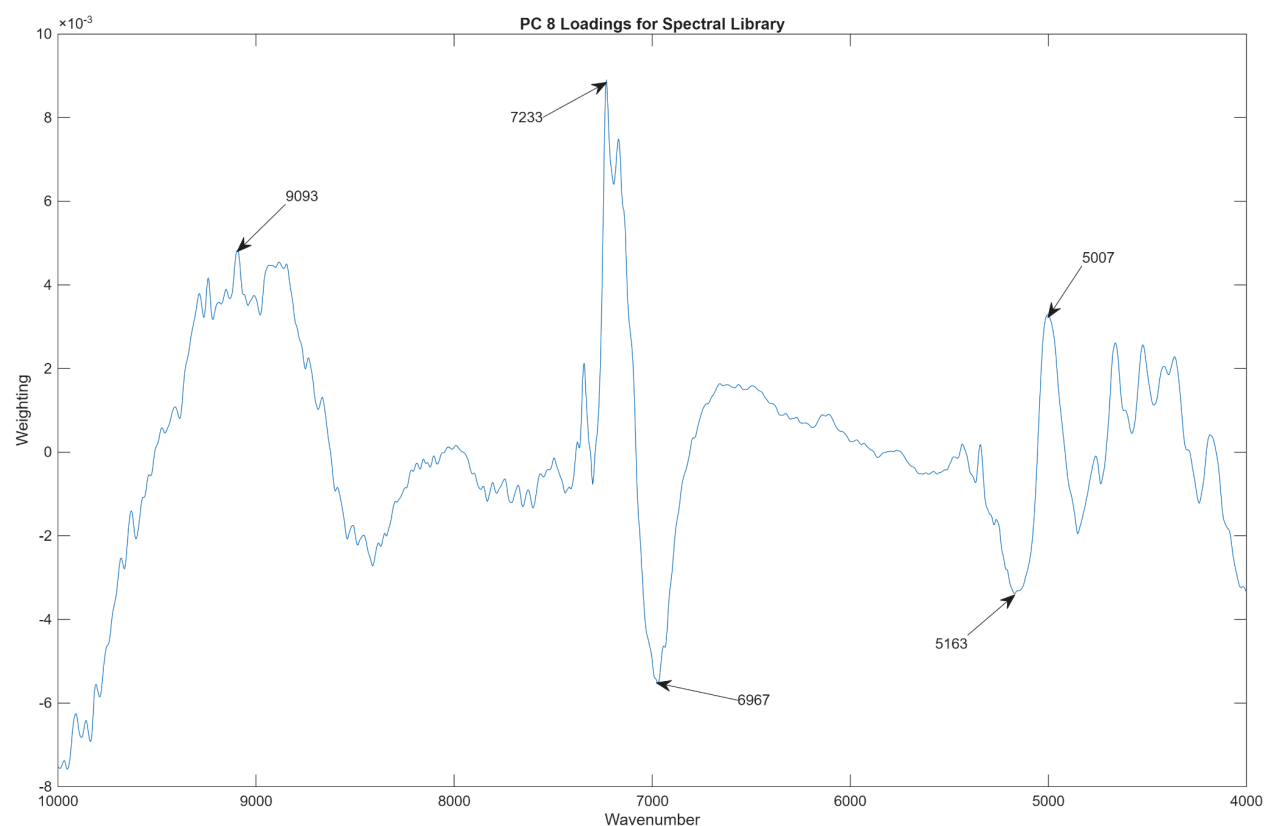


Figure 19. The loadings spectrum for PC 8 of the spectral library. Notable peaks are marked at 5007, 5163, 6967, 7233, and 9093 cm^{-1} .

An analysis of the loadings spectrum for the eighth principal component (PC 8) displayed in [Figure 19](#) reveals notable spectral peaks located at 5007, 5163, 6967, 7233, and 9093 cm^{-1} . Applying standard near-infrared spectra-structure correlations to these specific wavenumbers identifies the functional groups driving this highly targeted dimension of variance. The highest wavenumber peak at 9093 cm^{-1} is situated in the higher overtone region and is typically

associated with the third overtone of C-H stretching vibrations originating from the aliphatic hydrocarbon framework of the molecule. The peak at 7233 cm^{-1} falls within the first overtone region and also characteristically corresponds to the first overtone of C-H stretching modes. The absorption at 6967 cm^{-1} is located in the first overtone region as well, but is generally associated with the first overtone of an O-H or N-H stretching vibration, pointing toward fluctuations in the complex amine and amide environments of the drug or potentially trace moisture. The peak at 5163 cm^{-1} is widely recognized as an O-H combination band coupling stretching and deformation modes, acting as a sensitive marker for variations in hydration or trace water content within the lyophilized matrix. Finally, the peak at 5007 cm^{-1} lies near the boundary of the combination region and is frequently assigned to the second overtone of a carbonyl (C=O) stretch, though it can also overlap with complex N-H combinations.

[Figure 19](#) is an essential inclusion in the report because it mathematically isolates and physically characterizes an extremely subtle, orthogonal source of inter-lot variance that emerges deep within the high-dimensional spectral hyperspace. According to the comprehensive inter-lot variance analysis detailed in [Table 2](#), PC 8 accounts for only 0.04% (0.0004) of the total spectral variation across the library. While the primary principal components capture broad, batch-level structural inconsistencies, explicitly documenting a higher-order component like PC 8 provides the rigorous statistical baseline necessary to evaluate minor multidimensional outliers and subtle probability-density perturbations. By mapping the exact wavenumbers driving this specific component, the figure allows investigators to detect minute, highly localized chemical anomalies, demonstrating that the manufacturing process experiences multiple, independent sources of physical and chemical instability rather than a single, uniform mode of failure.

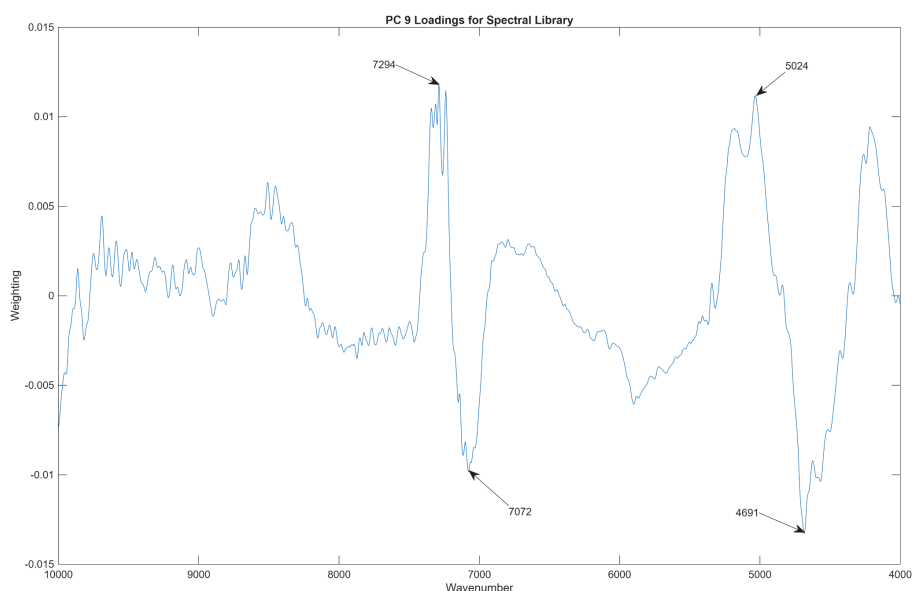


Figure 20. The loadings spectrum for PC 9 of the spectral library. Notable peaks are marked at 4691, 5024, 7072, and 7294 cm^{-1} .

An analysis of the PC 9 loadings spectrum in [Figure 20](#) reveals notable spectral peaks located at 4691, 5024, 7072, and 7294 cm^{-1} . Applying standard near-infrared spectra-structure correlations to these specific wavenumbers identifies the functional groups driving this ultimate dimension of variance. The peak at 7294 cm^{-1} falls within the first overtone region and is characteristically associated with the first overtone of C-H stretching vibrations originating from the aliphatic hydrocarbon framework of the aztreonam molecule. The absorption at 7072 cm^{-1} is also located in the first overtone region and generally corresponds to the first overtone of an O-H or N-H stretching vibration, which points toward trace moisture or fluctuations in the complex amine and amide environments. The peak at 5024 cm^{-1} sits near the boundary of the combination region and is frequently assigned to O-H combination bands or the second overtone of a carbonyl (C=O) stretch. Finally, the peak at 4691 cm^{-1} lies within the combination region and typically represents complex combinations of C-H or N-H stretching and deformation modes.

[Figure 20](#) is an essential inclusion in the report because it mathematically isolates and physically characterizes the final, most subtle orthogonal source of inter-lot variance evaluated deep within the high-dimensional spectral hyperspace. According to the comprehensive inter-lot variance analysis detailed in [Table 2](#), PC 9 accounts for a mere 0.01% (0.0001) of the total spectral variation across the library. While the primary principal components capture broad, batch-level structural inconsistencies, explicitly documenting this final higher-order component provides the complete, rigorous statistical baseline necessary to evaluate the smallest multidimensional outliers. By mapping the exact wavenumbers driving this specific component, the figure allows investigators to detect minute, highly localized chemical anomalies, demonstrating that the manufacturing process is subjected to multiple, independent sources of physical and chemical instability all the way down to the absolute mathematical limits of the model.

Conclusion

Aztreonam for injection (Fresenius Kabi) is a sterile, nonpyrogenic, sodium-free lyophilized, off-white to slightly yellow solid containing approximately 780 mg of arginine per gram of aztreonam. Following constitution, the drug is indicated for the treatment of specific infections caused by susceptible Gram-negative microorganisms via intramuscular or intravenous use. Through near-infrared spectrometric screening, significant intra-lot variability was observed in the chemical and physical matrix of this drug product. An initial principal component analysis of localized vial samples revealed considerable variation, where the spectral data points wandered around a central axis to form a distinct toroidal pattern. This ring-like geometry indicates cyclical oscillatory dynamics or an unstable mathematical average state within the underlying manufacturing system. Furthermore, analysis of higher-order principal components isolated extreme multidimensional outliers, with specific vials situated up to 5.6 standard deviations away from the center of the data cluster. The trajectory of these outliers diverging in multiple

distinct directions confirms that the drug is being subjected to multiple, independent sources of physical and chemical instability rather than a single mode of processing failure.

Expanding the evaluation to a comprehensive inter-lot analysis of 152 vials across 12 different lots demonstrated that these inconsistencies persist across the broader manufacturing scale. The primary principal components of the library showed the spectral data dividing into two major, statistically distinct groups, with one group exhibiting a notably higher number of outliers. This asymmetric distribution provides strong statistical evidence that certain batches of the drug were subjected to a much looser state of process control or experienced highly variable intermittent disturbances. Additionally, projections in the higher-order dimensions (PCs 7, 8, and 9) revealed that an entire manufacturing lot of the drug separated to form a completely different group, uncovering systemic, batch-level deviations from the standard production profile.

To rigorously validate these visual clustering observations, the Fast Son of BEST (FSOB) algorithm was utilized to evaluate nonparametric probability-density contours. A quantile-quantile (QQ) plot of the empirical cumulative distribution function plotted against the theoretical cumulative distribution function displayed a distinct, sharp step-like shape. This mathematically confirms the presence of distinct subpopulations and demonstrates a highly significant departure from a single, uniform multivariate distribution. The algorithm officially detected two major groups within the spectral library ($r_{tm}=0.99$, $r_{ts}=0.88$, $p=0.02$). Ultimately, these spectrometric results do not prove an excess level of impurities or adulteration. However, they suggest that the manufacturing process may have been operating outside of a state of process control. Additional investigation is needed.

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