

Rapid Quality Screening of Intra-lot Variability of Acetazolamide Using Near-Infrared Spectrometry

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RAPID COMMUNICATION

Abstract

Acetazolamide for Injection, USP is a sterile, preservative-free carbonic anhydrase inhibitor primarily indicated for the treatment of acute mountain sickness, fluid volume overload associated with heart failure, and elevated intraocular pressure in glaucoma. This research utilized Fourier transform near-infrared spectrometry (FT-NIR) to evaluate the intra-lot variability of 12 vials of Mylan Institutional Acetazolamide 500 mg. While all sampled vials appeared visually identical as white to yellowish-white powders, FT-NIR analysis identified significant spectral heterogeneity, particularly in the range of 4200 to 5200 cm^{-1} . Statistical analysis using the Bootstrap Error-Adjusted Single-sample Technique (BEST) and Principal Component Analysis (PCA) identified vial 6 as a prominent statistical outlier. This vial exhibited a distinct absorbance peak at 4851 cm^{-1} , which was suggested to be a shift from a peak at 5173 cm^{-1} and was positioned 4.03 standard deviations from the center of the lot cluster in multidimensional spectral hyperspace. These results suggest that the manufacturing process may have been operating outside of a state of process control. Given the ongoing national shortages of

acetazolamide as of January 2026, such quality assurance screenings are critical for maintaining supply chain transparency and ensuring the reliability of medication used in evolving clinical protocols.

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 (FT-NIR) 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

Acetazolamide for Injection, USP is a sterile preservative free, non-pyrogenic agent used for intravenous administration. The visual appearance is white to faintly yellowish white powder, and each vial contains 500 mg of active ingredient ([Mylan, 2025](#)).

Acetazolamide is an inhibitor of an enzyme called carbonic anhydrase that has an effect on control of fluid and electrolyte balance that is abnormally present in heart failure and epilepsy that is mainly caused by fluid overload issue ([Mylan, 2025](#)).

[Figure 1](#) is a photo of the Mylan Institutional acetazolamide drug product. The lot number was 7610176.



Figure 1. Acetazolamide 500 mg Mylan institutional Lot 7610176, Exp. 10/2026. A total of 12 vials were scanned and then re-scanned twice (rescan 1 and rescan 2).

Background

[Acetazolamide](#) is an irreversible carbonic anhydrase inhibitor that has diuretic effect by reducing ion secretion at renal tubule and increasing renal excretion of sodium, potassium, bicarbonate, and thereby water. The diuretic therapeutic effect is used in various clinical conditions that includes acid-base balance and body fluid regulation. It also has an anti-seizure effect by inhibiting the carbonic anhydrase in the central nervous system that prevents abnormal or excessive discharge from CNS neurons. The FDA approved uses include acute mountain sickness or high-altitude cerebral edema, general body fluid volume overload due to heart failure, and elevated intraocular pressure with chronic or acute open-angle glaucoma ([Mylan, 2025](#)).

Recalls

As of early 2026, there have been no recent, wide-scale mandatory recalls for Mylan's acetazolamide for injection issued by the FDA or international bodies like the EMA. However, there was a significant regulatory and safety event centered around this product in late 2020, primarily driven by independent quality testing. A [Citizen's Petition](#) ([Citizen's Petition, 2020](#)) was filed in September 2020 requesting the recall of a number of lots of Mylan and Hikma acetazolamide.

Shortages

As of January 2026, there are ongoing shortages of acetazolamide for injection affecting both the United States and international markets. The situation is dynamic, with supply levels varying significantly between manufacturers.

The American Society of Health-System Pharmacists (ASHP) currently lists acetazolamide for injection as a "current shortage" as of January 20, 2026 ([ASHP Drug Shortages List, 2026](#)). Viatris (Mylan Institutional) currently has the 500 mg vials (NDC 67457-0853-50) available. Hikma Pharmaceutical's product is available but on allocation, meaning they are limiting shipments to existing customers to manage inventory. Avet Pharmaceuticals reported 500 mg vials on backorder with no firm resupply date provided in the most recent updates. X-Gen Pharmaceuticals product remains on backorder ([Becker's Hospital Review, 2025](#)).

Current Research

Recent research (2024–2026) into acetazolamide for injection has been dominated by its expanding role in cardiology and neuro-ophthalmology, moving beyond its traditional use in glaucoma. The landmark ADVOR (Acetazolamide in Decompensated Heart Failure With Volume Overload) trial remains a primary catalyst for current study. Recent post-hoc analyses published in late 2024 and 2025 demonstrate that adding intravenous acetazolamide (500 mg daily) to standard loop diuretic therapy significantly increases the likelihood of successful decongestion—defined as the absence of volume overload signs within three days—by approximately 46%. Scientists are now investigating its "chloride-preserving" potential, noting that while loop diuretics often deplete serum chloride, acetazolamide helps restore it, potentially preventing the diuretic resistance commonly seen in acute heart failure patients. ([Van den Eynde](#))([Umeh, 2024](#))

In the realm of neurology and ophthalmology, 2024–2025 research has utilized the "acetazolamide challenge" to establish new biomarkers for retinal health. Specifically, studies using optical coherence tomography (OCT) have shown that systemic injection of acetazolamide induces measurable subretinal acidification and dehydration, which serves as a diagnostic stress test for photoreceptor mitochondrial efficacy. Furthermore, ongoing clinical trials in 2025 are exploring the drug's use in Idiopathic Normal Pressure Hydrocephalus (iNPH) to improve gait and cognitive function by modulating cerebrospinal fluid production. Despite these clinical advances, the medical community is currently navigating a significant supply challenge; as of January 2026, acetazolamide for injection remains on the national drug shortage list (ASHP) due to manufacturing backlogs, complicating the implementation of these new evidence-based protocols ([Virhammar, 2025](#))([Berkowitz, 2024](#)).

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)(Yoon,).

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, 1993](#)).

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, 2023](#))([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.

Result and analysis

Intra-Lot Analysis

[Figure 2](#) shows the results of 36 FTNIR raw spectra of 12 vials of Acetazolamide 500 mg. Each vial was scanned 3 times, moving the vial exposed to the beam each time. The result revealed all samples appeared superficially similar with visual inspection, with some differences in the spectra mainly appearing from 4200 to 5200 cm^{-1} .

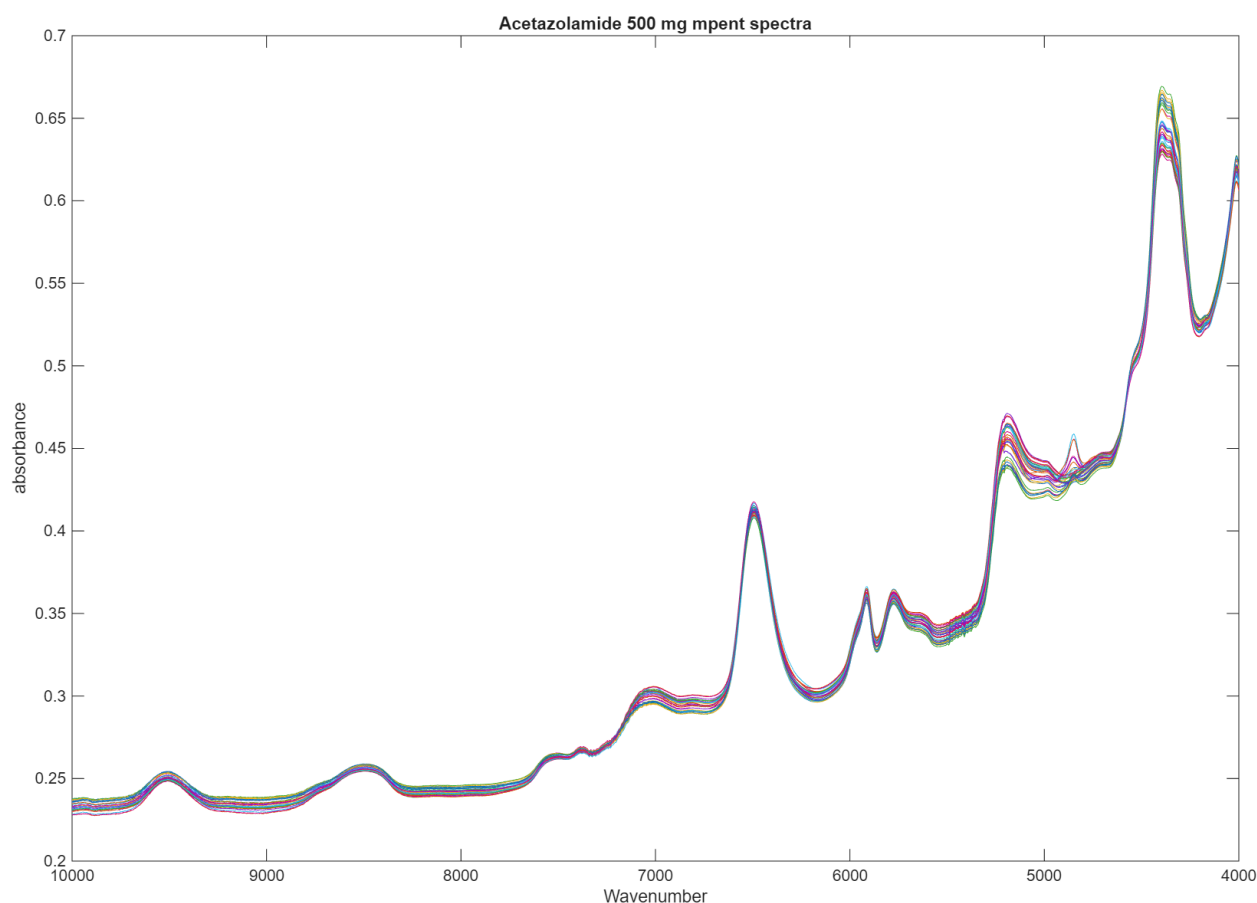


Figure 2. Acetazolamide spectra from lot 7610176, with a total 36 spectra for the 12 sampled vials including rescans 1 and 2. Differences between the spectra mainly appear from 4200 to 5200 cm^{-1} .

[Figure 3](#) presents a magnified view of the FTNIR spectra for the acetazolamide lot, specifically focusing on the wavenumber range between 4200 and 5600 cm^{-1} . This detailed visualization is critical because it exposes intra-lot inconsistencies that remain hidden during a broad visual inspection. The importance of this figure lies in its ability to highlight that a few vials within the lot possess a distinct absorbance peak at 4851 cm^{-1} that is missing in the majority of the other sampled vials. This spectral variation seems to stem from a peak shift from 5173 cm^{-1} to 4851 cm^{-1} , a finding that ultimately helped classify vial 6 as a statistical outlier located 4.03 standard deviations from the center of the lot cluster. Consequently, [Figure 3](#) serves as a piece of evidence for the Drug Quality Task Force to detect potential manufacturing defects or chemical variations within a single medication lot. The peak at 4851 cm^{-1} was also noted in the Citizen's Petition to FDA ([Citizen's Petition, 2020](#)) to recall some lots of acetazolamide, and the peak had a larger area in the petition.

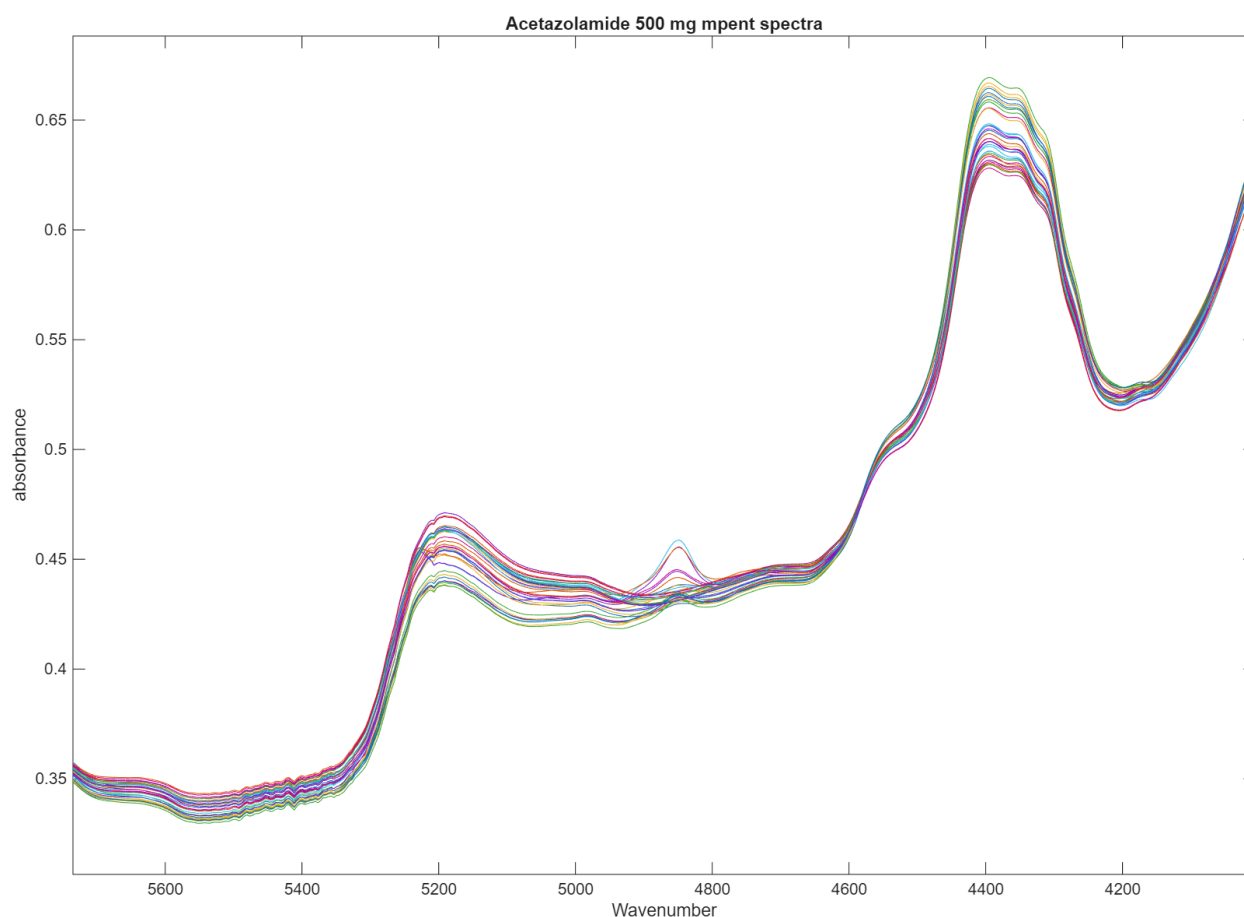


Figure 3. A zoom-in on the acetazolamide spectra in [Figure 2](#) covering the wavenumber range from 4200 to 5600 cm^{-1} . A few vials show a peak at 4851 cm^{-1} that most of the vials do not have.

[Figure 4](#) shows the difference spectrum between vial 6 and the intralot center (mean). Specifically, the interesting spectral variations are at the following wave numbers: 4851.3, 5173.2, 6398.8, 8256.6, and 9501.6 cm^{-1} . This specific visualization is important because it clearly isolates and highlights the interesting spectral variations that distinguish this particular vial from the rest of the lot. The difference spectrum reveals a prominent peak at 4851 cm^{-1} , which appears to be the result of a significant peak shift from 5173 cm^{-1} to 4851 cm^{-1} . By providing this granular look at the spectral anomalies, [Figure 4](#) serves as a primary diagnostic tool for confirming the intra-lot variability that caused vial 6 to be identified as a statistical outlier, and can help to identify the chemical(s) responsible.

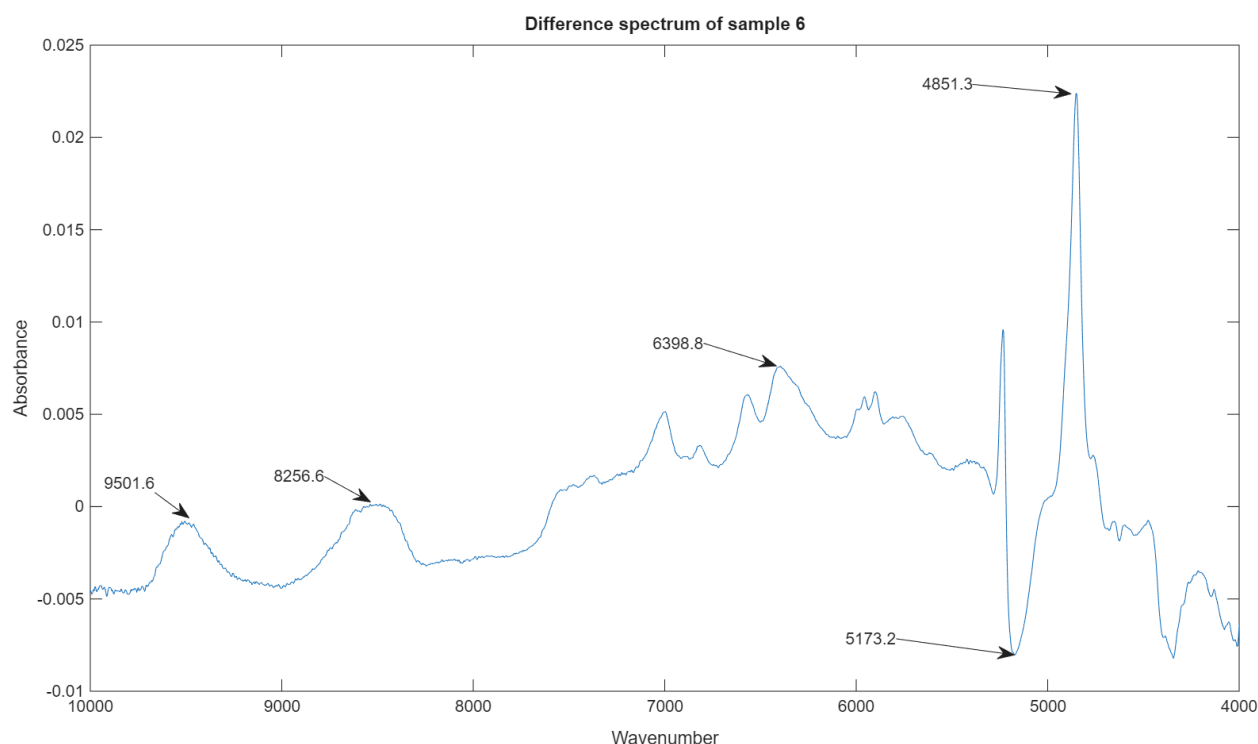


Figure 4. The difference spectrum between vial 6 and the mean of the lot 7610176 spectra shown in [Figure 2](#). The peak at 4851 cm^{-1} shows up clearly and appears to be the result of a peak shift from 5173 to 4851 cm^{-1} . Smaller peaks also appear at about 5200 cm^{-1} as well as at 6399 , 8257 , and 9502 cm^{-1} .

The statistical significance of vial 6 being 4.03 standard deviations (SD) from the lot center is substantial, as it identifies the vial as a clear outlier within the intra-lot analysis. In multivariate statistical screening, distances are calculated using the Bootstrap Error-Adjusted Single-sample Technique (BEST), which maps samples in an asymmetric, nonparametric central 68% confidence interval in spectral hyperspace. While samples within an ordinary 3 SD limit generally require multiple instances to be flagged as unusual with statistical significance, a single sample appearing beyond this threshold (like vial 6) indicates a significant departure from the expected chemical or physical profile of the lot. This degree of separation, visualized in [Figure 5](#), suggests that the spectral variations observed in this vial are not merely random noise but represent a distinct subcluster or a specific quality defect.

[Figure 5](#) presents a principal component (PC) score plot that maps the vials from lot 7610176 within a three-dimensional spectral hyperspace defined by the first three principal components. This visualization is important because it mathematically demonstrates the degree of intra-lot variability by showing how individual samples cluster together based on their spectral signatures. Most significantly, the plot identifies vial 6 as a major statistical outlier, positioned 4.03 standard deviations from the center of the main cluster. This spatial separation confirms that the spectral anomalies detected in [Figure 3](#) and [Figure 4](#) represent a statistically significant deviation from the rest of the batch, providing the objective evidence needed to flag potential quality defects within the drug product.

Principal Components of Intralot Vials

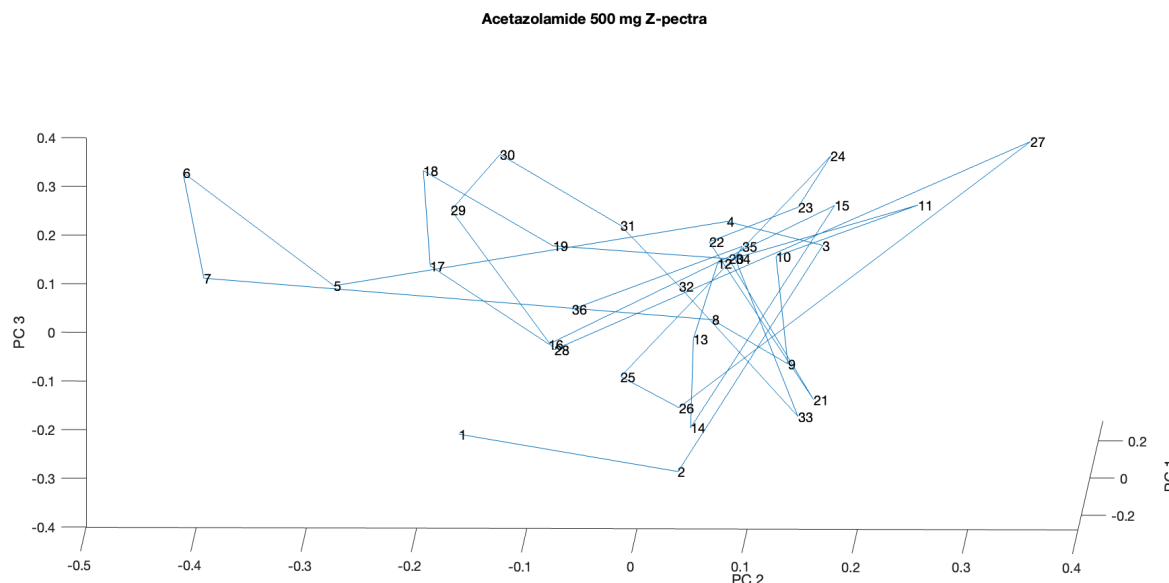


Figure 5. PC score plot of vials from lot 7610176 shown in [Figure 2](#). Vial 6 on the left side of the plot is 4.03 SDs from the center of the cluster of the other vials in the lot.

[Figure 6](#) displays a principal component score plot for the acetazolamide lot using the fourth, fifth, and sixth principal components. This visualization is important because principal component analysis (PCA) works by decomposing data variance orthogonally, where the first few components capture the largest variations while subsequent components reveal residual structures. While PCs 1–3 might highlight the most dominant differences, such as the 4.03 standard deviation outlier status of vial 6, examining PCs 4–6 allows researchers to detect more subtle intra-lot subclusters or unusual samples that may not be obvious in the primary dimensions. For instance, PC 5 specifically highlights spectral variation at wavenumbers like 4859.9 and 9510.28 cm^{-1} , which are critical for detecting unusual samples within the training set. By analyzing these higher-order components, the Drug Quality Task Force can more accurately describe odd shapes in spectral hyperspace and identify factors like low-level contamination or instrumental drift that might otherwise be overlooked.

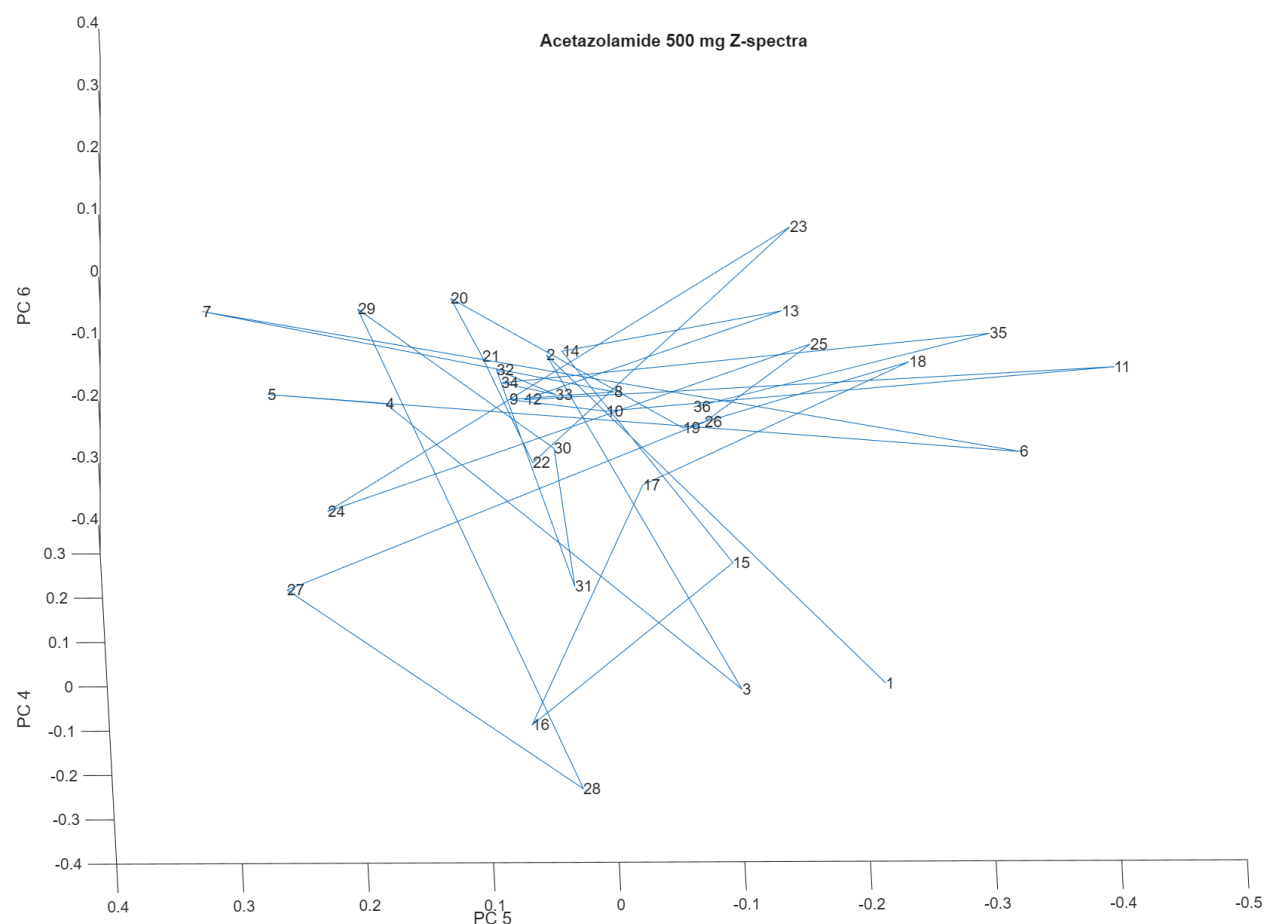


Figure 6. Another PC score plot (PCs 4, 5, and 6) of spectra of the vials from lot 7610176 shown in [Figure 2](#).

[Figure 7](#) presents a PC score plot for the acetazolamide lot using the seventh, eighth, and ninth principal components to visualize the relationships between the sampled vials in higher-dimensional spectral hyperspace. This visualization is significant because it highlights vial 19 as a notable outlier, showing a distinct displacement from the central cluster primarily along the PC 7 and PC 9 axes. The importance of this specific separation lies in the spectral variations captured by these components: PC 7 highlights interesting variations at several wavenumbers, including 5230.08, 7867.61, and 9521.85 cm^{-1} , while PC 9 captures variations at 4605.4, 4732.65, 5233.93, 6367.61, 6996.14, 8619.54, and 9494.86 cm^{-1} . By appearing as an outlier in these specific dimensions, vial 19 exhibits chemical or physical signatures that differ from the main lot in ways distinct from the previously identified outlier, vial 6, which was most prominent in earlier PCs. This demonstration of multi-dimensional variability underscores the capability of the FSOB and BEST methods to detect unusual samples and subclusters—potentially caused by factors like moisture uptake or low-level contamination—even when they do not manifest as primary sources of variance.

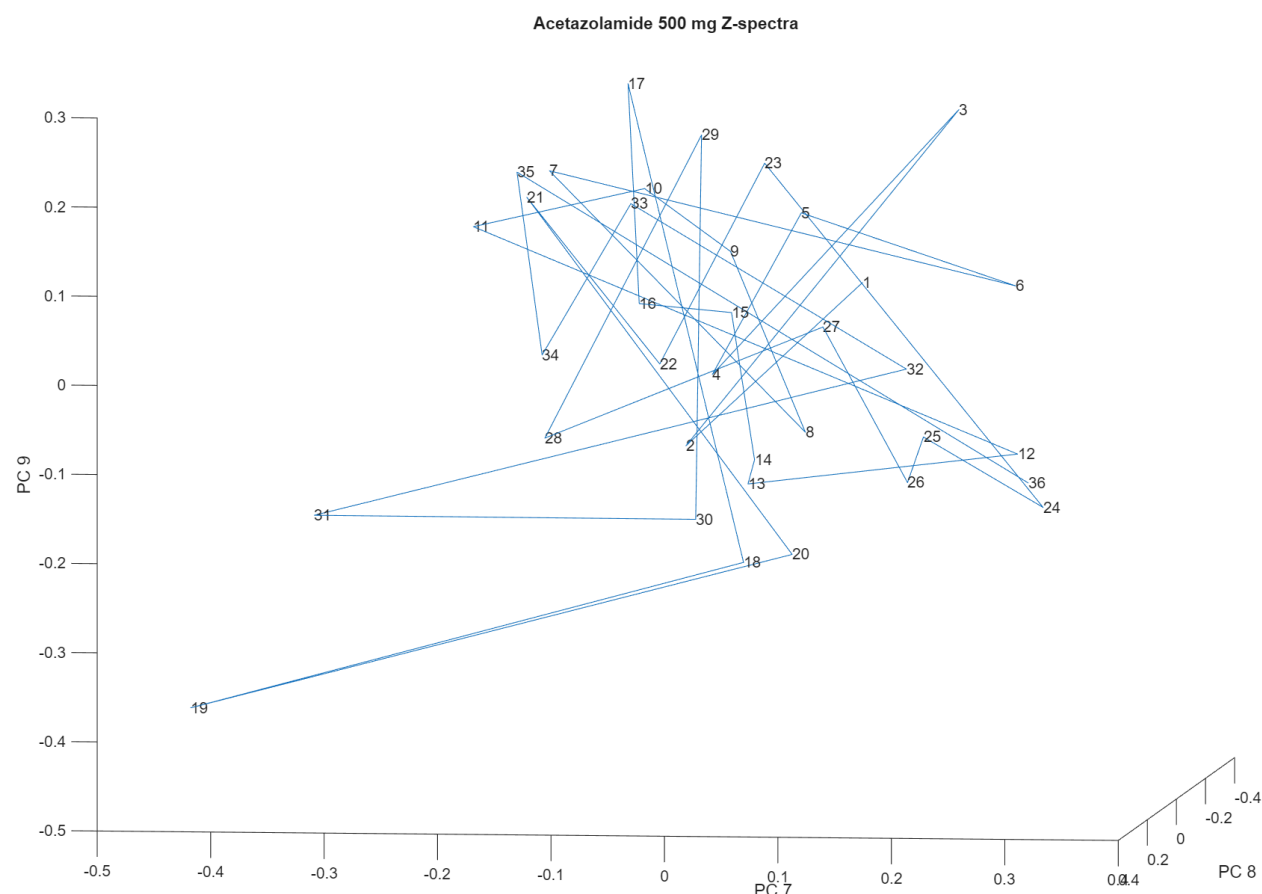


Figure 7. Another PC score plot (PCs 7, 8, and 9) of vials from lot 7610176 shown in [Figure 2](#).

PC Loadings Spectra

The loadings plots are essential for interpreting the results of the Principal Component Analysis (PCA) because they identify which specific wavenumbers contribute most to the variance seen in the score plots. Each Principal Component (PC) captures a different dimension of spectral variation: PC 1, for example, highlights variations at 4100.7, 6997.9, and 8341.8 cm^{-1} , while PC 5, which relates to the detection of unusual samples within the training set, emphasizes wavenumbers like 4859.9 and 9510.28 cm^{-1} . By mapping these weightings, researchers can link the statistical separation of an outlier, such as vial 6, back to physical or chemical phenomena occurring at those specific spectral points, such as the peak shifts identified in the difference spectrum. This allows the Drug Quality Task Force to transition from simply identifying that a vial is "different" to understanding the exact spectral regions and molecules where that difference originates.

[Figure 8](#) presents the principal component loadings spectrum for the first principal component (PC 1) of the acetazolamide spectral library. This visualization identifies specific wavenumbers, most notably 4100.7, 6997.9, and 8341.8 cm^{-1} , where the most significant spectral variations occur across the batch. Modeling such baseline variations within the principal components is

essential because factors like sample packing, particle-size distributions, moisture uptake, and instrumental drift can cause clusters in spectral hyperspace to vary significantly in shape and size. By accounting for these common variations, researchers can better differentiate between routine baseline fluctuations and actual quality defects or low-level contamination. This comprehensive modeling ensures that a result placing a point inside a cluster truly indicates membership rather than an overlap with a new, slightly different cluster created by environmental or instrumental factors.

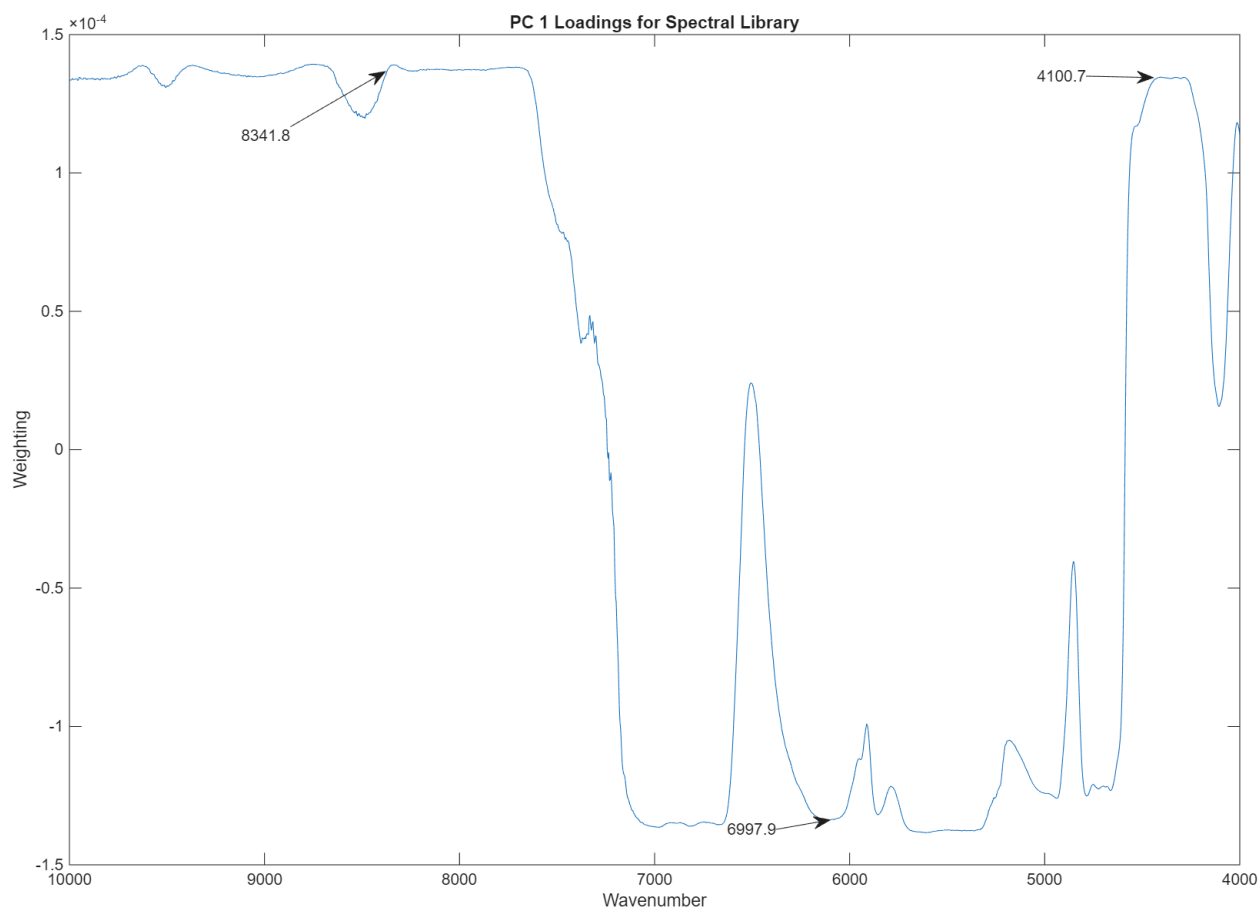


Figure 8. The principal component loadings spectrum for the first principal component of the batch of acetazolamide.

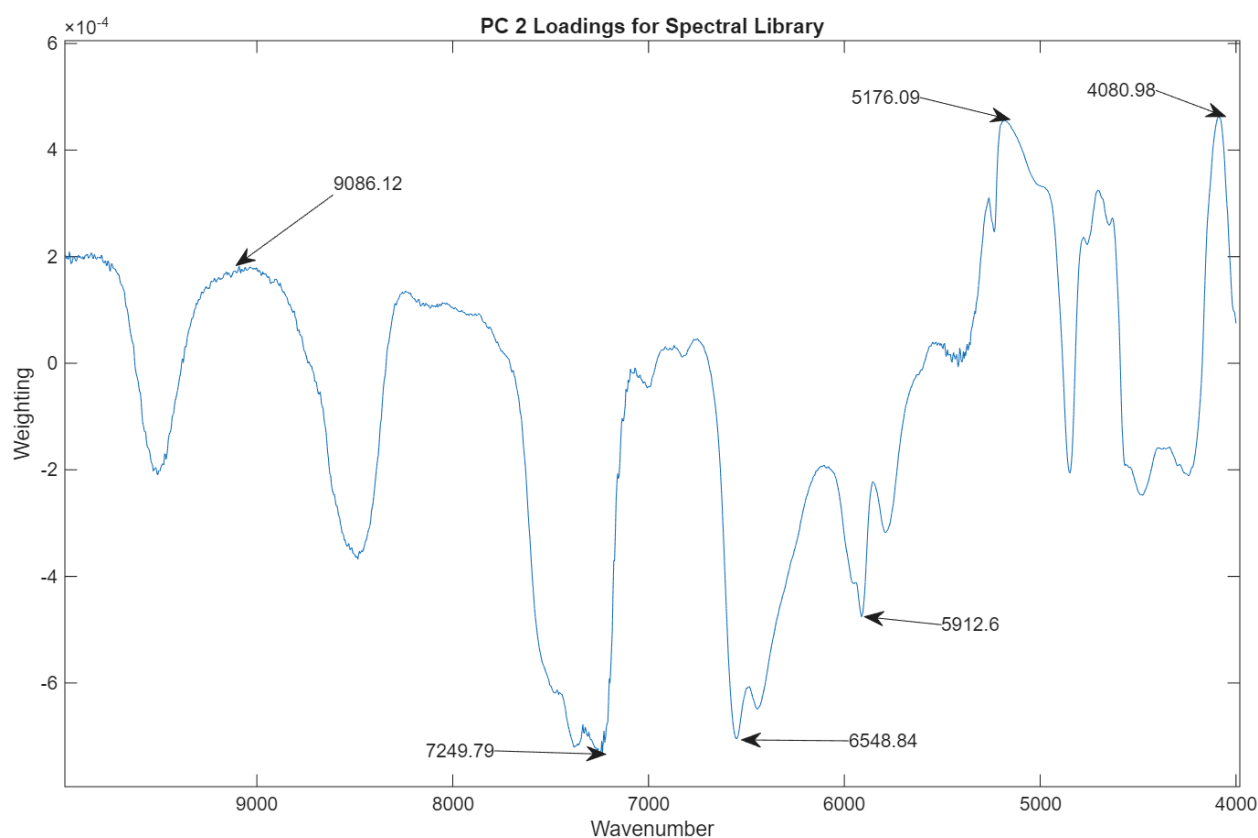


Figure 9. The principal component loadings spectrum for the second principal component of the acetazolamide.

[Figure 9](#) shows a spectral lot of the loadings for the second principal components (PC2) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4080.98, 5176.09, 5912.6, 6548.84, 7249.79, and 9086.12 cm^{-1} . While PC 1 often captures broad baseline variations such as sample packing or particle-size distributions, PC 2 begins to reveal additional, more specific sources of chemical differences. This is important because accounting for multiple sources of variance allows the model to more accurately define the boundaries of a "normal" cluster in spectral hyperspace. By characterizing these additional chemical signatures, the system can better distinguish between routine manufacturing fluctuations and potential quality defects, such as low-level contamination or moisture uptake, that might otherwise be masked by baseline noise.

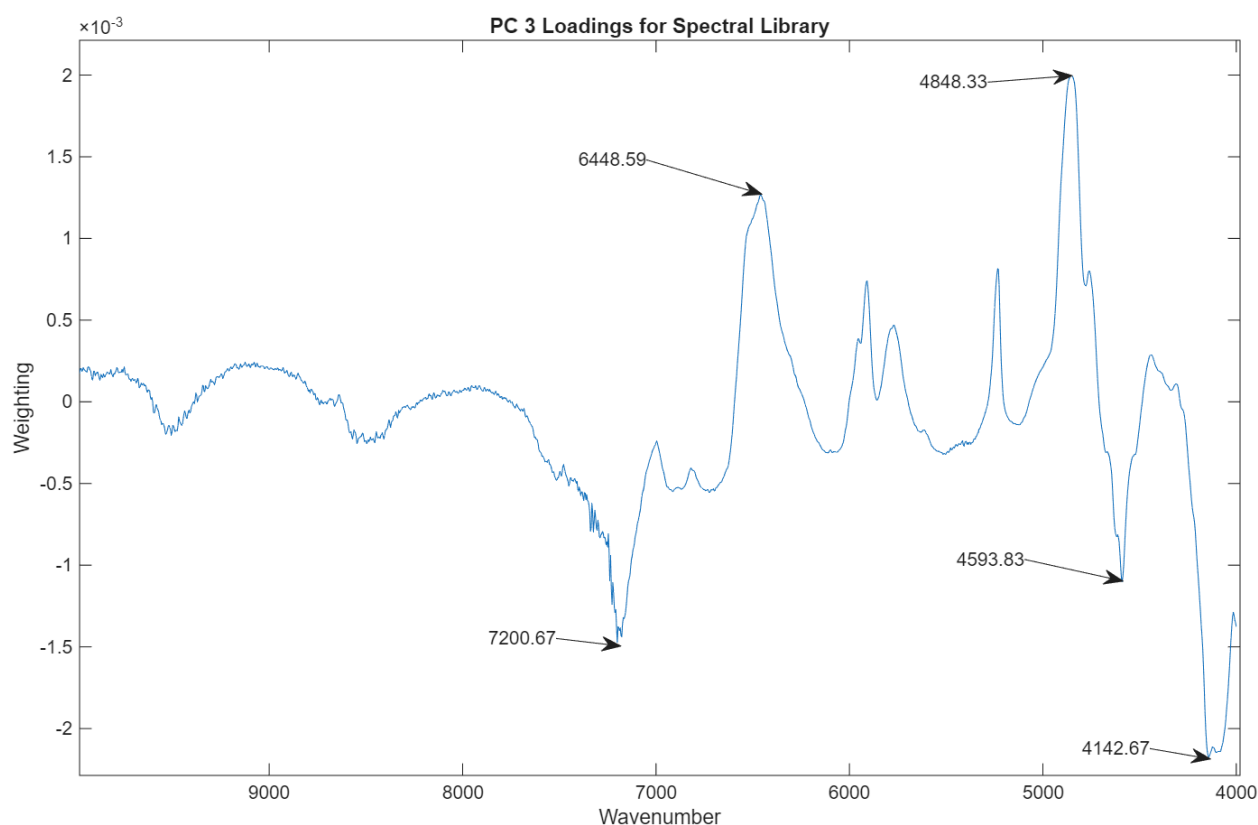


Figure 10. The principal component loadings spectrum for the third principal component of the acetazolamide.

[Figure 10](#) shows a spectral plot of the loadings for the third principal components (PC3) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4142.67, 4593.82, 4848.33, 6448.59, and 7200.67 cm^{-1} . The importance of PC 3 capturing more specific chemical differences rather than baseline variations lies in its ability to isolate unique anomalies that characterize outliers like vial 6. Specifically, the loading at 4848.33 cm^{-1} in [Figure 10](#) corresponds closely to the anomalous peak at 4851 cm^{-1} identified in the difference spectrum of vial 6. By focusing on these chemical shifts instead of broad baseline fluctuations like sample packing or instrumental drift, the analysis can effectively differentiate between routine manufacturing noise and actual quality defects or chemical inconsistencies within a medication lot.

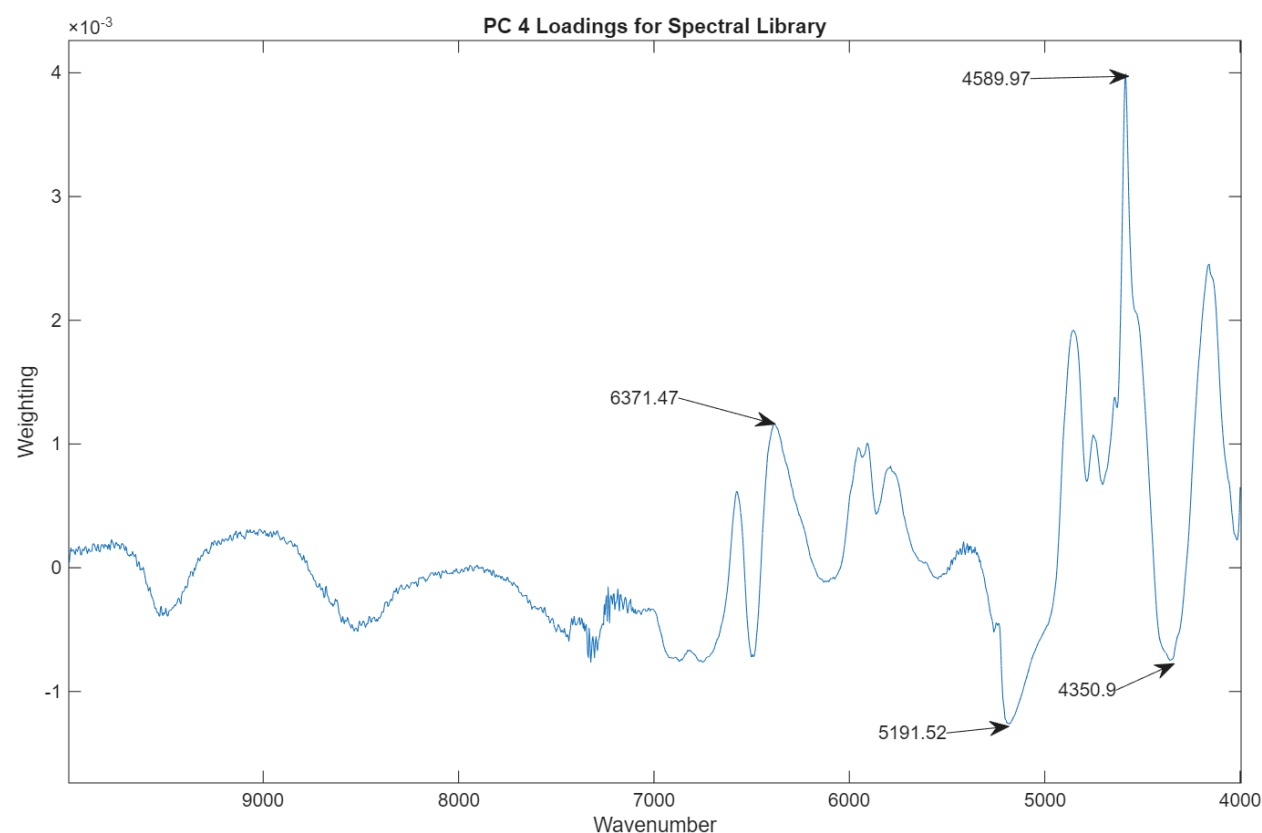


Figure 11. The principal component loadings spectrum for the fourth principal component of the acetazolamide.

[Figure 11](#) shows a spectral lot of the loadings for the fourth principal components (PC4) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4350.9, 4589.97, 5191.52, and 6371.47 cm^{-1} . The figure is important because it continues the orthogonal decomposition of variance, capturing chemical and physical differences that were not accounted for by the first three principal components. Notably, at the high wavenumber end of the spectrum near 10000 cm^{-1} , a small amount of noise is beginning to appear, suggesting that PC 4 is starting to resolve lower-magnitude signals or instrumental limitations compared to the more dominant baseline variations seen in PC 1. By modeling these more subtle variations, the analysis can better define the multidimensional shape of the lot's spectral cluster, which is essential for accurately identifying subclusters caused by moisture uptake, drift, or low-level contamination. The peaks from chemistry are still much larger than the peaks from the noise.

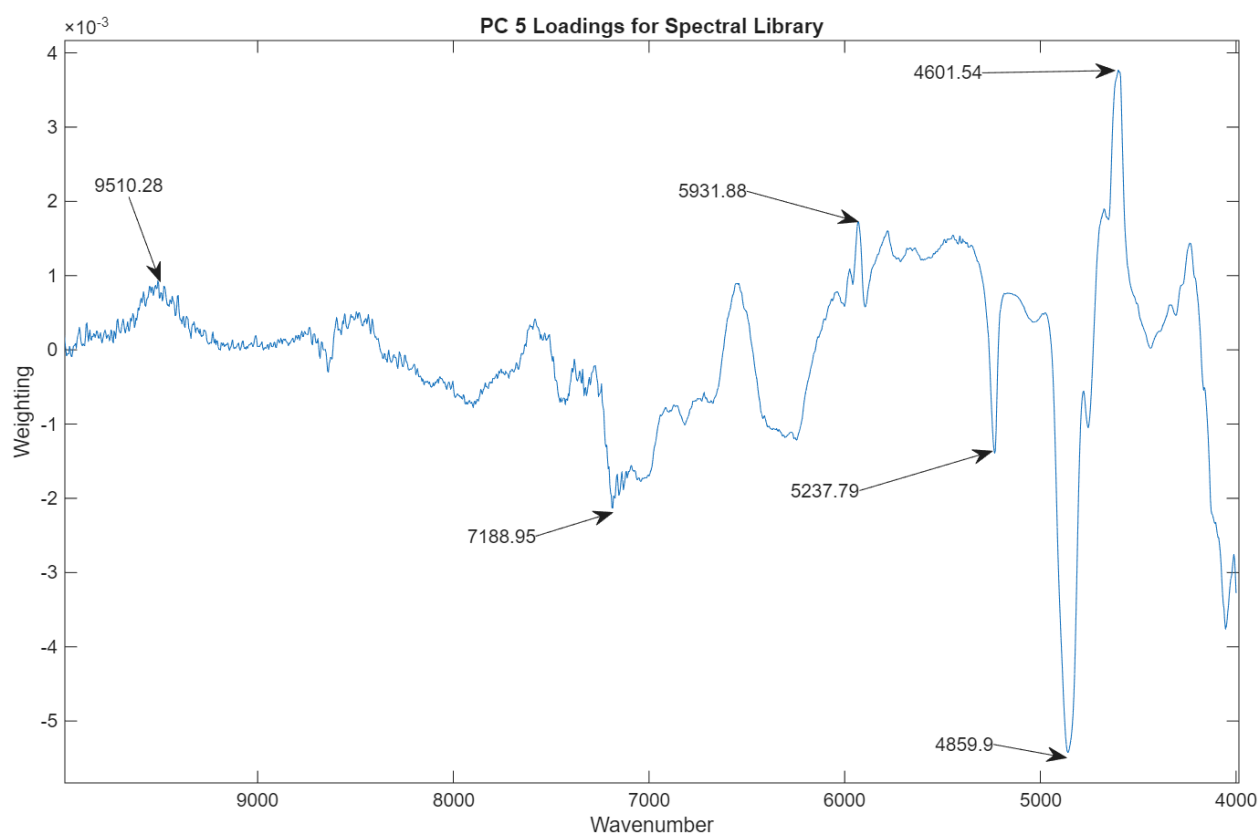


Figure 12. The principal component loadings spectrum for the fifth principal component of the acetazolamide.

[Figure 12](#) shows a spectral plot of the loadings for the fifth principal components (PC5) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4601.54, 4859.9, 5237.79, 5931.88, 7188.95, and 9510.28 cm^{-1} . This visualization is important because it captures more subtle chemical and physical differences that were not fully accounted for by the first three principal components, thereby helping to refine the multidimensional shape of the spectral cluster. As variance decomposition continues orthogonally, a small amount of noise becomes visible at the high wavenumber end of the spectrum, indicating that PC 4 is beginning to resolve lower-magnitude signals or instrumental limitations that were masked by the dominant baseline variations in earlier components. By modeling these nuanced variations, the analysis enhances the ability to detect unusual samples and subclusters—such as those caused by low-level contamination or moisture uptake—that might otherwise be overlooked within the ordinary 3 SD limits.

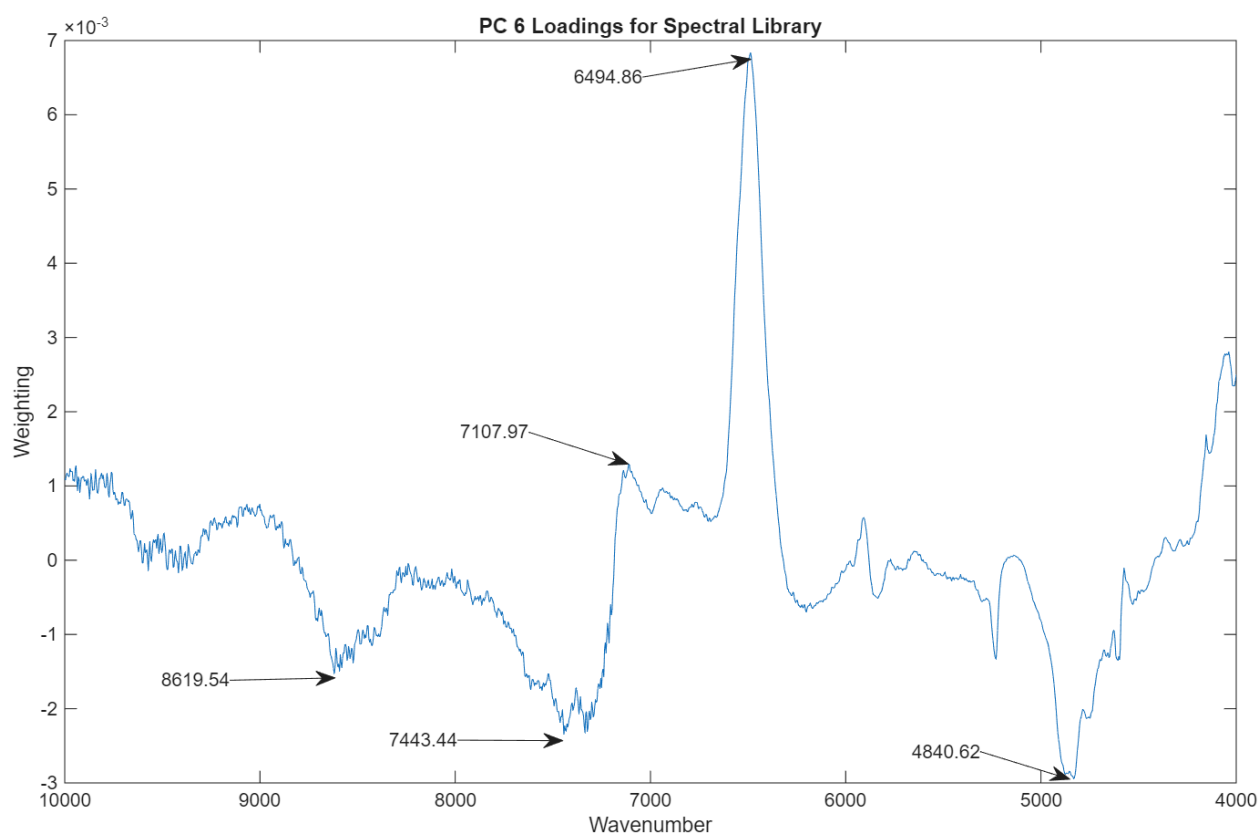


Figure 13. The principal component loadings spectrum for the sixth principal component of the acetazolamide.

[Figure 13](#) shows a spectral lot of the loadings for the sixth principal components (PC6) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4840.62, 6494.86, 7107.97, 7443.44, and 8619.54 cm^{-1} . As the analysis progresses through higher-order principal components, the loadings continue to capture more nuanced chemical and physical differences within the lot that were not resolved by the earlier, more dominant components. By identifying these specific regions of variance, researchers can more effectively detect unusual samples and subclusters caused by factors such as low-level contamination, moisture uptake, or instrumental drift, even when those samples appear within the ordinary 3 SD limit of the training set center.

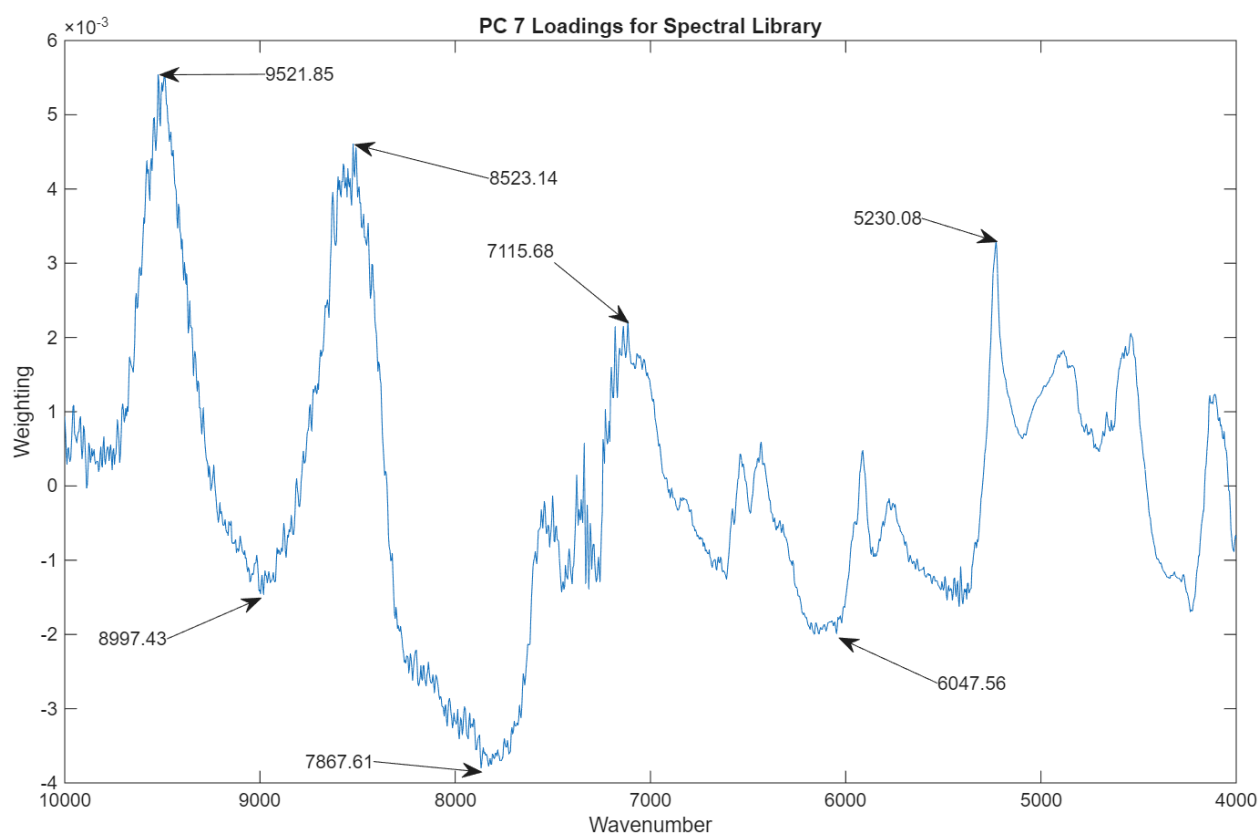


Figure 14. The principal component loadings spectrum for the seventh principal component of the acetazolamide.

[Figure 14](#) shows a spectral lot of the loadings for the seven principal components (PC7) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 5230.08, 6047.56, 7115.68, 7867.61, 8523.14, 8997.43, and 9521.85 cm^{-1} . Notably, large peaks appear in the high wavenumber region, specifically at 8523.14 and 9521.85 cm^{-1} , that significantly dwarf the noise that is observed in that area. By capturing these prominent variations, PC 7 continues to resolve nuanced chemical and physical differences within the lot, allowing researchers to detect unusual samples and subclusters that may be caused by factors like moisture uptake or low-level contamination. This level of detail is essential for the Drug Quality Task Force to accurately map the spectral hyperspace and identify potential manufacturing defects or chemical variations within a single medication lot.

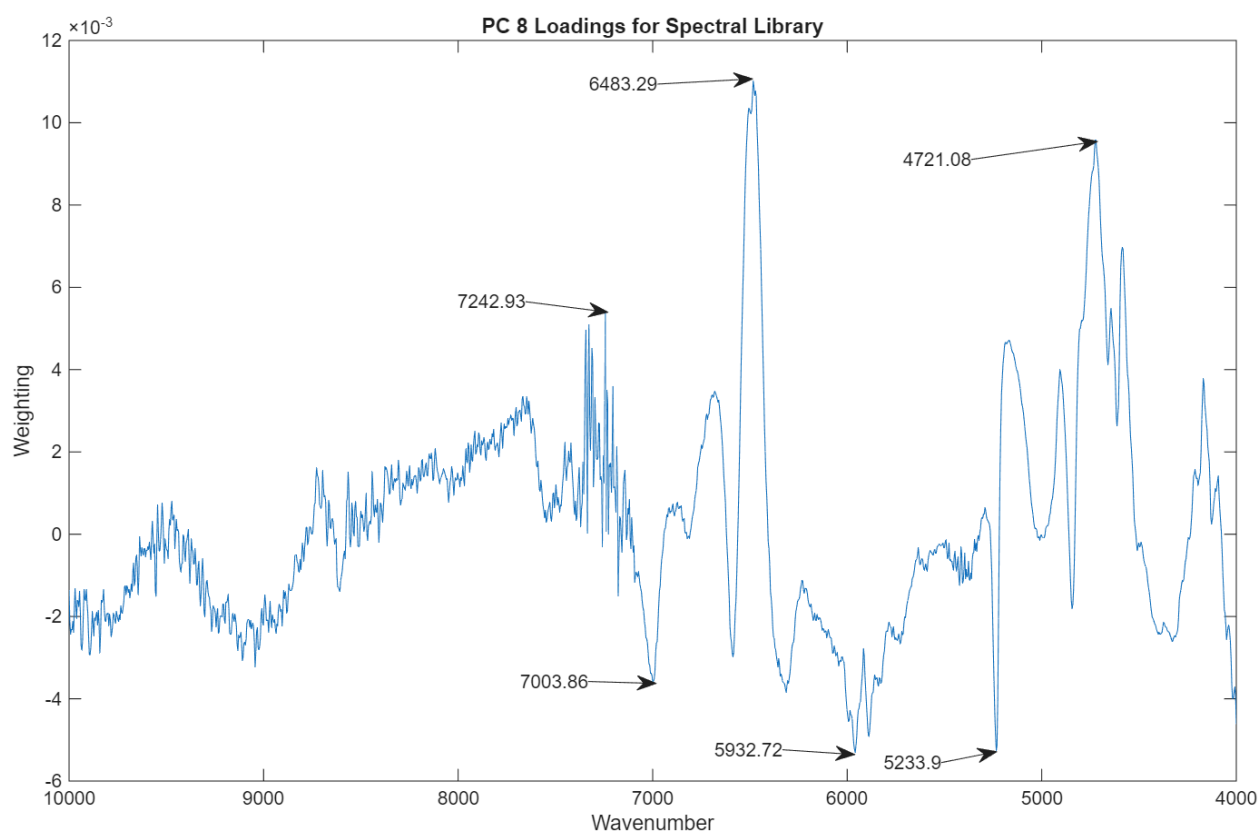


Figure 15. The principal component loadings spectrum for the eighth principal component of the acetazolamide.

[Figure 15](#) shows a spectral plot of the loadings for the eight principal components (PC8) of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4721.06, 5233.9, 5932.72, 6483.29, 7003.86, and 7242.93 cm^{-1} . As the eighth orthogonal component, it resolves nuanced chemical and physical differences that were not captured by the preceding seven components. The identification of these specific variation points is important for the Drug Quality Task Force as it allows for the detection of unusual samples or subclusters within spectral hyperspace, which can be triggered by low-level contamination, instrumental drift, or moisture uptake. By modeling these increasingly subtle variations, the analysis provides a more comprehensive diagnostic profile of the medication lot, ensuring that even minor intra-lot inconsistencies are identified.

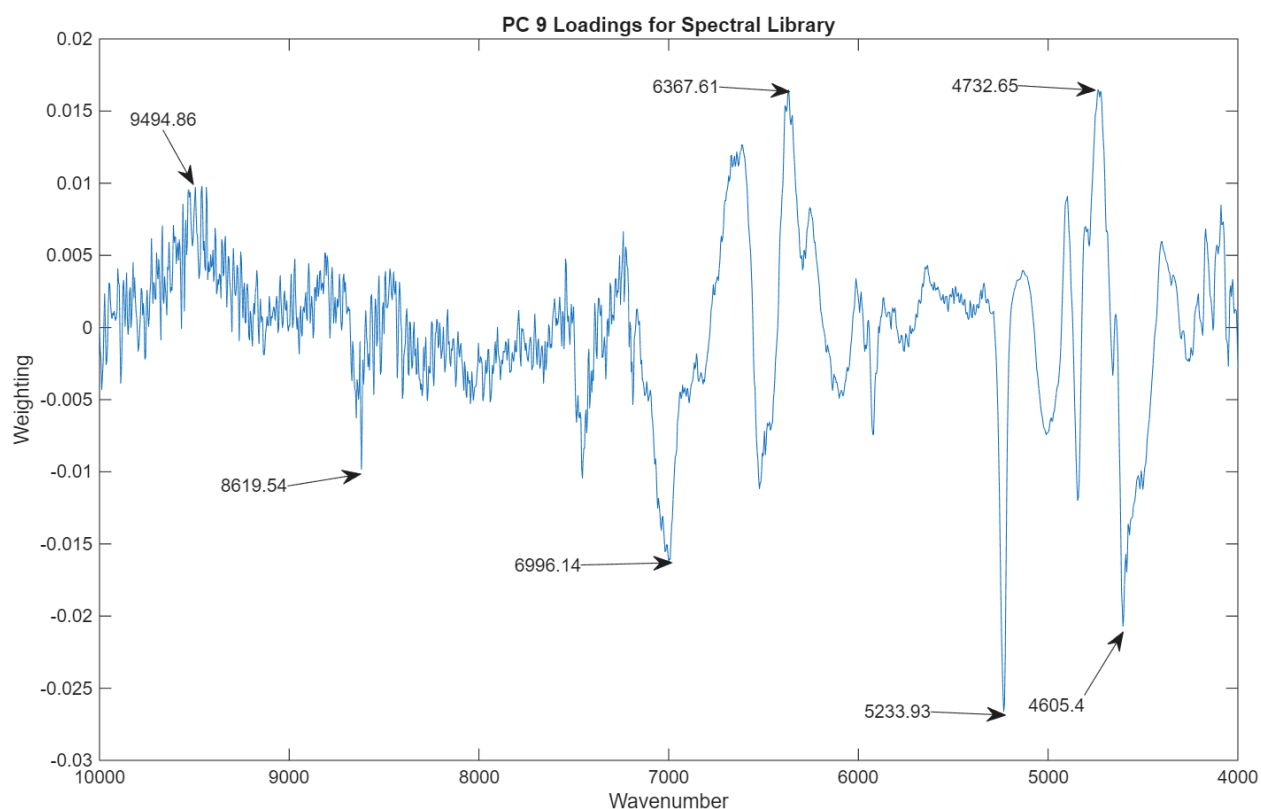


Figure 16. The principal component loadings spectrum for the ninth principal component of the acetazolamide.

[Figure 16](#) shows a spectral plot of the loadings for PC9 of the acetazolamide library. The plot specifically highlights interesting spectral variation at the following wave numbers: 4732.65, 4605.4, 5233.93, 6367.61, 6996.14, 8619.54, and 9494.86 cm^{-1} . This visualization is critical because it represents a high-dimensional stage of the variance decomposition where the magnitude of the identified chemical differences is becoming comparable in size to the instrumental and environmental noise. Capturing these subtle, lower-magnitude signals is important for precisely defining the boundaries of spectral clusters in hyperspace, allowing for the detection of unusual samples or subclusters that might be caused by factors like minor moisture uptake, instrumental drift, or low-level contamination. By modeling these variations at the threshold of noise, the analysis provides a comprehensive diagnostic framework that ensures even the most nuanced intra-lot inconsistencies are identified, supporting the overarching goal of improving pharmaceutical supply chain transparency.

Conclusion

This project utilized FTNIR spectrometry to perform rapid quality screening on Mylan Institutional Acetazolamide 500 mg (Lot 7610176). Although the twelve sampled vials appeared superficially similar and consistent with the expected visual appearance of a white to faintly yellowish powder, the FTNIR analysis revealed significant intra-lot heterogeneity that visual inspection could not detect. Magnifying the spectral range between 4200 and 5600 cm^{-1} , a distinct peak at 4851 cm^{-1} was discovered in a subset of vials, which further diagnostic analysis suggested was a spectral peak shift from 5173 cm^{-1} . These variations provided the

mathematical basis for identifying vial 6 as a statistical outlier, positioned 4.03 standard deviations from the center of the lot cluster in the principal component score plot. Such findings underscore the mission of the University of Kentucky's Drug Quality Task Force (DQTF) to hold manufacturers accountable for Good Manufacturing Practice (GMP) requirements and to enhance transparency within the pharmaceutical supply chain. This work is especially important given the ongoing national shortages of acetazolamide for injection as of January 2026, which also complicates the implementation of new evidence-based clinical protocols in cardiology and neuro-ophthalmology.

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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