

Spectrometric Assessment of Intralot and Interlot Variability in Mycophenolate Mofetil for Injection Using Near-Infrared Spectrometry and Nonparametric Subcluster Detection

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RAPID COMMUNICATION

Abstract

Mycophenolate Mofetil for Injection is an antimetabolite immunosuppressant supplied as a sterile, lyophilized powder containing 500 mg of active drug per single-dose vial for intravenous administration. As a prodrug of mycophenolic acid, it selectively and reversibly inhibits inosine

monophosphate dehydrogenase to block de novo guanosine nucleotide synthesis and suppress lymphocyte proliferation, carrying boxed warnings for embryofetal toxicity, serious opportunistic infections, and malignancies. Nondestructive Fourier transform near-infrared spectrometry coupled with principal component analysis and the Bootstrap Error-Adjusted Single-sample Technique was utilized to evaluate intralot and interlot spectral consistency across drug vials. Intra-lot analysis of a single 15-vial batch (lot 2515203) revealed heterogeneity and outliers, with individual vials deviating up to 11.6 multidimensional standard deviations from the main cluster center. Interlot evaluation of a 464-vial spectral library spanning 18 distinct manufacturing lots demonstrated persistent two-group subclustering, with Principal Component 1 accounting for only 87.82% of total spectral variance. Nonparametric FSOB density estimation confirmed statistically significant subclustering across the multi-lot library ($p = 0.02$), dividing the sample set into two distinct groups (14.2% vs. 85.8%). While these findings do not establish active ingredient adulteration or excessive impurities, the observed intralot and interlot spectral variations indicate that the manufacturing process may have operated outside an ideal state of statistical process control, underscoring the value of PAT monitoring in health-system quality assurance.

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, 2023a](#)). 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

Mycophenolate Mofetil for Injection (manufactured by Kindos Pharmaceuticals Co., Ltd. and distributed by Meitheal Pharmaceuticals Inc.) is an antimetabolite immunosuppressant supplied as a sterile, lyophilized powder containing 500 mg of mycophenolate mofetil per single-dose vial for intravenous administration. As a prodrug of mycophenolic acid (MPA), it acts as a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), selectively blocking the de novo pathway of guanosine nucleotide synthesis essential

for T- and B-lymphocyte proliferation. It is FDA-indicated in combination with other immunosuppressants for the prophylaxis of organ rejection in adult and pediatric patients (3 months of age and older) receiving allogeneic kidney, heart, or liver transplants. The drug carries boxed warnings for embryofetal toxicity associated with increased risks of first-trimester pregnancy loss and congenital malformations as well as heightened susceptibility to serious opportunistic infections and an increased risk of malignancies, particularly lymphoma and skin cancers. The most common adverse reactions (>20%) reported in clinical trials include diarrhea, leukopenia, infection, vomiting, hypertension, and peripheral edema ([U.S. National Library of Medicine, 2026](#))

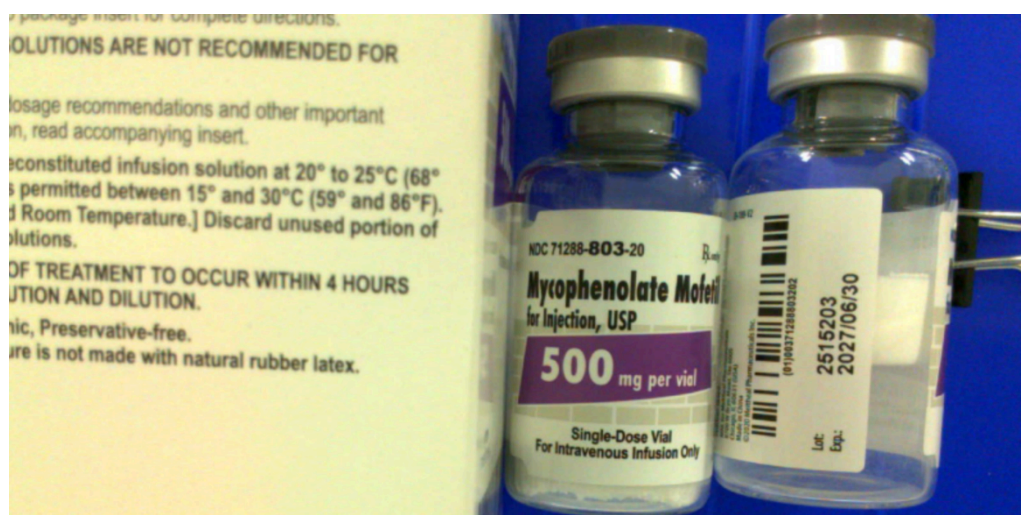


Figure 1. Vials of Mycophenolate Mofetil for Injection drug product.

[Figure 1](#) is a photo of the drug product including some packaging.

The 18 lot numbers making up the spectral library of Kindos Pharmaceuticals Mycophenolate Mofetil were 2215008, 2215009, 2315024, 2315034, 2415201, 2415202, 2415203, 2415206, 2415207, 2415210, 2415211, 2415212, 2415213, 2415214, 2415215, 2515201, 2515203, and 2515204.

Background

Recent studies

Mycophenolate mofetil (MMF) continues to serve as a cornerstone immunosuppressive agent within modern clinical practice, spanning solid organ transplantation, rare neuroinflammatory disorders, nephrology, and autoimmune rheumatological conditions. Recent clinical data published between 2024 and 2026 have significantly refined our understanding of MMF's efficacy, safety, dosing paradigms, and steroid-sparing capabilities. This section evaluates the latest peer-reviewed clinical trials, multicenter cohort studies, and meta-analyses to provide an updated summary of MMF's contemporary therapeutic role.

Neurological and Rare Neuroinflammatory Disorders

In the management of myasthenia gravis (MG)([Narayanaswami, 2024](#)), recent high-impact data from the prospective PROMISE-MG cohort study demonstrated that MMF and azathioprine (AZA) achieve comparable clinical efficacy and quality-of-life improvements. Notably, MMF exhibited a more favorable safety profile with significantly fewer serious adverse events compared to AZA, although clinicians must remain cognizant of its teratogenic profile in patients of childbearing potential. These findings are strongly supported by a 2024 real-life multicenter experience published in Brain Sciences ([Di Stefano, 2024](#)), which highlighted that MMF offers faster symptom control, minimal manifestation status achievement, and superior long-term tolerability relative to conventional AZA regimens.

Beyond myasthenia gravis, MMF has demonstrated critical efficacy in managing rare neuroinflammatory conditions like Neuromyelitis Optica Spectrum Disorders (NMOSD) and Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disorders (MOGAD)([Zhou, 2025](#)). A 2025 Chinese multicenter cohort study confirmed that while monoclonal antibodies provide optimal relapse prevention, MMF and rituximab are substantially more effective than tacrolimus or azathioprine positioning MMF as a premier off-label option in resource-limited clinical settings. Concurrently, recent evidence supports high-dose MMF as a potent strategy to reduce the annualized relapse rate in adult relapsing MOGAD, while separate investigations validate its utility as a dual induction and maintenance agent in adult primary central nervous system vasculitis ([John, 2025](#)).

Nephrology: Lupus Nephritis and IgA Nephropathy

The therapeutic paradigm for severe glomerulopathies has been heavily enriched by recent literature focusing on lupus nephritis (LN) and Immunoglobulin A Nephropathy (IgAN)([Hou, 2023](#)). In LN, systematic reviews and randomized clinical trials reinforce MMF's position as a preferred alternative to cyclophosphamide for induction therapy, achieving equivalent or superior remission rates alongside a profoundly reduced incidence of severe toxicities, leukopenia, and gastrointestinal distress ([Feng, 2024](#)). For diffuse proliferative lupus nephritis (DPLN),

combination protocols involving MMF and corticosteroids have led to marked reductions in proteinuria and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores, corroborated by post-treatment histological normalization.

In IgA Nephropathy, the landmark 2024 randomized clinical trial published in JAMA Network Open established that MMF combined with standard supportive care significantly slows down kidney disease progression in progressive IgAN cases compared to supportive care alone ([Wadei, 2024](#)). Meta-analyses corroborate these findings, indicating that MMF—particularly when combined with corticosteroids—reduces major adverse kidney events (MAKE) and mitigates renal function decline. This combination is especially effective among Asian populations, providing a vital therapeutic option where traditional cyclophosphamide-based regimens present unfavorable toxicity thresholds.

Solid Organ Transplantation and Precision Dosing Paradigms

Within solid organ transplantation, recent 2024 data have heavily concentrated on modern combination immunosuppression, steroid avoidance, and precision dosing strategies. The multicenter SAILOR study demonstrated that a steroid-avoidance regimen incorporating low-dose tacrolimus and MMF is highly feasible, safe, and effective up to two years post-transplantation in immunologically low-risk renal transplant recipients. Additionally, the real-world multicenter experience reported in 2024 by Wadei and colleagues revealed that physician-directed MMF dose reduction during the first post-transplant year is a common and safe practice that does not adversely affect graft survival, allograft inflammation, or donor-specific antibody (DSA) formation, while mitigating severe hematologic side effects.

Conversely, a separate trial emphasized that MMF significantly suppresses vaccine-induced antibody responses across a broad spectrum of routine vaccinations in kidney transplant recipients, demanding tailored preventative healthcare strategies.

From a regulatory and pediatric pharmacology perspective, 2024 marked significant milestones with formal reviews validating MMF for the prophylaxis of organ rejection in pediatric heart and liver transplant recipients, using model-informed precision dosing to successfully navigate unique childhood pharmacokinetic hurdles. Furthermore, novel protocols within haploidentical hematopoietic cell transplantation (HCT) using post-transplant cyclophosphamide indicate that a lower, weight-based MMF dose is associated with superior relapse-free and progression-free survival outcomes, highlighting the critical shift from rigid, fixed-dose administration to individualized, weight-modeled therapies ([Elmariah, 2024](#))([Ritter, 2024](#)).

Rheumatology, Systemic Sclerosis, and Autoimmune Hepatitis

In rheumatology, compelling evidence published in 2025 in ACR Open Rheumatology formally positioned MMF as the first-line standard of care for diffuse cutaneous systemic sclerosis (SSc)([White, 2025](#)), outperforming methotrexate and corticosteroids in optimizing skin and long-term lung outcomes. This clinical preference was mirrored in the INSIST trial for systemic

sclerosis-related interstitial lung disease (SSc-ILD), where MMF maintained its status as the foundational preferred agent due to its deep-seated safety track record, even though tacrolimus demonstrated non-inferiority ([Ge, 2025](#)).

Additionally, first-line utilization of MMF in dermatomyositis-associated interstitial lung disease has been shown to induce complete normalization of pulmonary function tests and complete resolution of severe dyspnea, showing excellent long-term patient tolerance.

Finally, a critical 2025 systematic review and meta-analysis published in Gastroenterology Research evaluated MMF against azathioprine in autoimmune hepatitis (AIH)([Khan, 2025](#)). The study revealed that MMF achieved an exceptionally high biochemical remission rate of 88.57% compared to only 53.64% in the AZA cohort, yielding a striking odds ratio of 7.81. While MMF exhibits a documented rate of initial GI intolerance requiring careful management, its high therapeutic efficacy renders it an ideal alternative first-line option or a highly successful second-line choice for patients who fail or exhibit intolerance to standard azathioprine protocols ([Ali, 2025](#)).

Collectively, peer-reviewed clinical studies from 2024 to 2026 reinforce mycophenolate mofetil's expanding status as an adaptable and highly potent immunomodulator. Whether through optimizing long-term survival in solid organ transplant recipients via steroid-sparing combination regimens, or by establishing new standards of care in rare rheumatological and neurological diseases, MMF consistently demonstrates a favorable efficacy-to-toxicity ratio. Future randomized controlled trials will further solidify individualized, model-informed precision dosing guidelines, maximizing therapeutic responses while avoiding hematological and infectious toxicities.

Recalls and Shortages

Mycophenolate mofetil (MMF) is a critical narrow therapeutic index (NTI) immunosuppressive agent relied upon for the prophylaxis of organ rejection in renal, cardiac, and hepatic transplant recipients, as well as for various off-label autoimmune indications. Given its narrow margin of safety and the catastrophic consequences of sub-therapeutic dosing (graft rejection) or over-dosing (severe toxicity), strict supply chain and manufacturing oversight is paramount. This regulatory section details documented drug shortages, significant drug recalls, and recent U.S. Food and Drug Administration (FDA) Warning Letters affecting the manufacturing, distribution, and safety monitoring of mycophenolate mofetil.

1. Market Availability and Drug Shortages

Mycophenolate mofetil is a high-demand compound processed by a variety of generic manufacturers. While individual generic firms have historically experienced isolated delays or capacity issues that mandated standard tracking within the American Society of Health-System

Pharmacists (ASHP) Current Drug Shortages tracking logs and the FDA Drug Shortage database, the drug is not currently facing systemic, nationwide market unavailability. Nonetheless, because the clinical success of transplant and autoimmune therapies depends heavily on uninterrupted supply, any individual regional or generic manufacturer shortage requires careful clinical substitution strategies to maintain generic and biosimilar therapeutic equivalence without disrupting steady-state pharmacokinetics.

2. Product Integrity and Manufacturing Recalls

Manufacturing deviations occasionally jeopardize the sterile and physical composition of mycophenolate mofetil formulations, necessitating targeted regulatory intervention. A major voluntary nationwide recall occurred when Par Pharmaceutical, Inc. issued a market withdrawal of Mycophenolate Mofetil for Injection, USP (specifically Lot AD812, expiry 09/2020, NDC 42023-172-04). The product, manufactured for Par Pharmaceutical by Gland Pharma Limited, was recalled at the hospital and retail pharmacy levels after a glass fragment foreign material was discovered in a vial following reconstitution ([FDA Safety Alert, 2019](#)).

The presence of a foreign particulate in an intravenous medication carries critical clinical risks. The FDA noted that administering glass particulates intravenously can induce severe local irritation or swelling. Far more critically, blood vessel blockage and life-threatening clotting can occur if the particulate enters systemic circulation. Although the recall concluded without documented patient adverse events, it underscores the persistent vulnerabilities in raw vial manufacturing and complex parenteral packaging lines.

3. FDA Warning Letters and Safety Program Evasion

The FDA actively monitors global supply chains and digital storefronts to prevent the distribution of misbranded, unapproved, or improperly managed lots of mycophenolate mofetil. Because MMF carries significant congenital risks, the FDA enforces a strict Risk Evaluation and Mitigation Strategy (REMS) program designed to reinforce proper clinical behaviors and safeguard patients of childbearing potential. Illegal online pharmacies frequently attempt to circumvent these protections.

In a formal Warning Letter issued to globalpharmacyplus.com (MARCS-CMS 631427), the FDA cited the platform for introducing unapproved and misbranded medications into interstate commerce, expressly naming products where the FDA-approved version is mandated to undergo a REMS program ([FDA Inspections and Warning Letters, 2022](#))([BioPharm International Compliance Records, 2019](#)). The regulatory agency emphasized that bypassing the REMS framework for an NTI drug like mycophenolate mofetil poses massive health risks to U.S.

consumers. Unregulated sourcing means the medications do not carry the same assurances of chemical safety, potency, or purity, risking sub-potency or chemical contamination that could quickly lead to sudden graft rejection or unintended systemic toxicities.

Maintaining the clinical integrity of mycophenolate mofetil regimens requires careful synchronization between medical providers, generic manufacturers, and regulatory bodies. While national market availability remains relatively stable, isolated particulate-related parenteral recalls and illegal internet distribution channels that bypass REMS safeguards emphasize the necessity of rigid procurement tracking. Clinical institutions must strictly source MMF from verified suppliers to verify structural purity, precise active ingredient concentrations, and absolute alignment with federally mandated safe-use infrastructures.

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, 2026a](#)).

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 ([Yoon, 2026b](#))([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

Intralot analysis

Smoothed spectra graphs

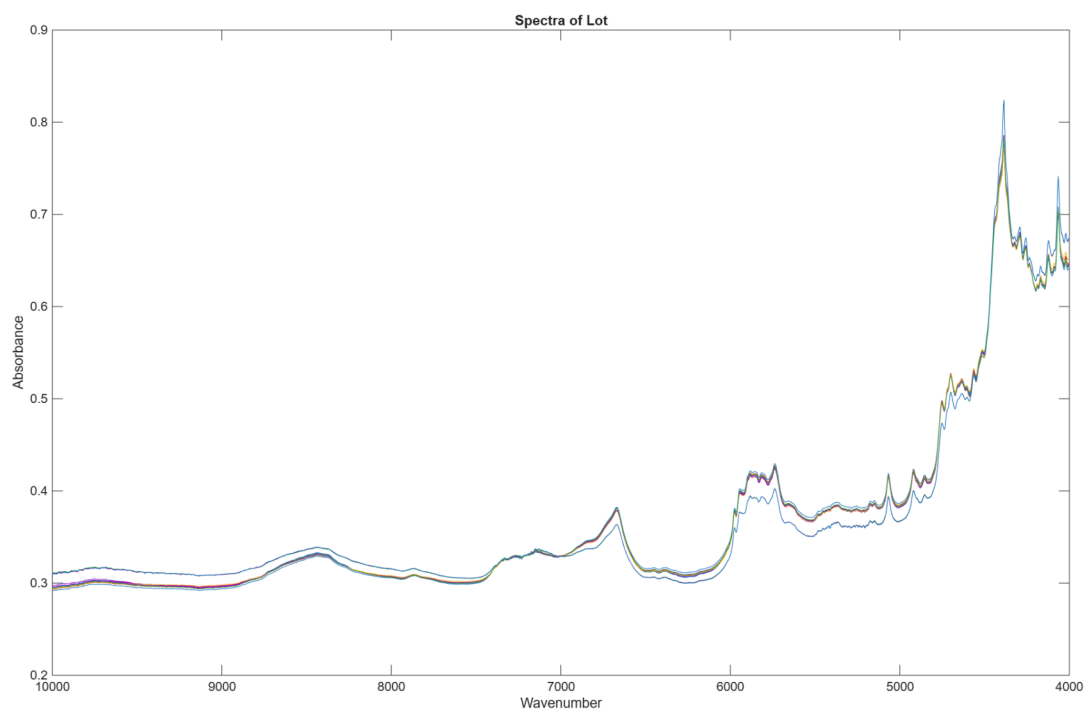


Figure 2. Spectra of 15 vials from lot 2515203 obtained non-invasively through the bottom of the vials.

[Figure 2](#) shows the Fourier transform near-infrared (FTNIR) spectra of 15 individual vials from a single batch (Lot 2515203) of Mycophenolate Mofetil for Injection. The spectra were collected non-invasively and non-destructively through the bottom of each vial and subjected to smoothing techniques. [Figure 2](#) serves as the baseline spectral data for analyzing consistency *within* a single manufacturing lot. The spectral data revealed variations among the vials subsequently quantified via Principal Component Analysis. These initial intralot spectral disparities provided the justification to expand the investigation and analyze a larger spectral library comprising 464 vials across 18 different drug lots.

PC Plots

[Figure 3](#) displays a principal component scatterplot of the Fourier transform near-infrared spectra for 15 vials selected from lot 2515203 of Mycophenolate Mofetil for Injection. By

projecting high-dimensional spectral data into principal component space, the plot visually reveals the spatial distribution and clustering behavior of the individual vials. The primary importance of [Figure 3](#) lies in its identification of significant intralot heterogeneity. Rather than showing a single uniform cluster, the pattern suggests that vials within the same manufacturing batch split into at least two distinct groups. Furthermore, the plot provides quantitative evidence of statistical outliers by measuring their distances from the center of the primary cluster in multidimensional standard deviations (SDs). Specifically, Vial 5 sits 5.1 SDs away, Vial 13 is 9.7 SDs away, Vial 14 is 9.6 SDs away, and Vial 15 reaches 11.6 SDs away from the main cluster center. The analysis accompanying the figure also notes that apparent visual proximity in PC space (such as the appearance of Vial 15 relative to Vials 13 and 14) can be an artifact of the viewing angle rather than true proximity in higher-dimensional hyperspace. Ultimately, the substantial distance metrics and evidence of subgrouping documented in [Figure 3](#) served as the critical empirical trigger that prompted the expansion of the investigation beyond a single lot to evaluate the broader 464-vial spectral library.

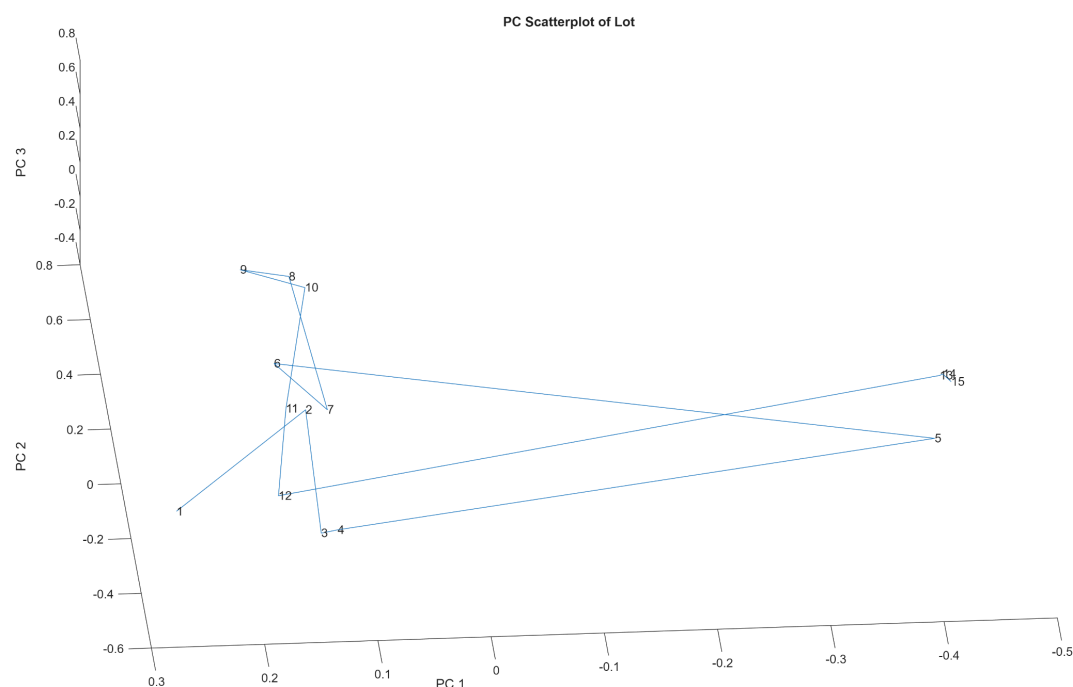


Figure 3. PC scatterplot of 15 vials from lot 2515203. The pattern suggests that there may be at least two groups in the data. Vial 5 is 5.1 multidimensional standard deviations (SDs) from the center of the other cluster, while vial 13 is 9.7 SDs away, vial 14 is 9.6 standard deviations away, and vial 15 is 11.6 standard deviations away from the center of the other vials. The difference in distances between vial 13 and 14, which seem close together, and to vial 15 is an artifact of the viewing angle. These distances were enough to trigger a closer examination of more lots of the drug.

In near-infrared multivariate statistical analyses, spectral data can split into separate clusters or subgroups due to a variety of physical, chemical, and operational factors within the sample or measurement environment. Physically, variations in sample packing, particle-size distributions,

or differences in the physical state of the lyophilized cake inside the vials can alter how light reflects and scatters, creating distinct spectral groupings. Chemically, subtle differences in active pharmaceutical ingredient or excipient concentrations, moisture uptake during storage, or low-level contamination can shift the spectral signatures enough to form a secondary cluster in hyperspace. While these spectral separations do not definitively prove the presence of adulteration or excessive impurities, they suggest that variations in the drug product or its preparation exist, suggesting that the manufacturing process may have operated outside an ideal state of process control.

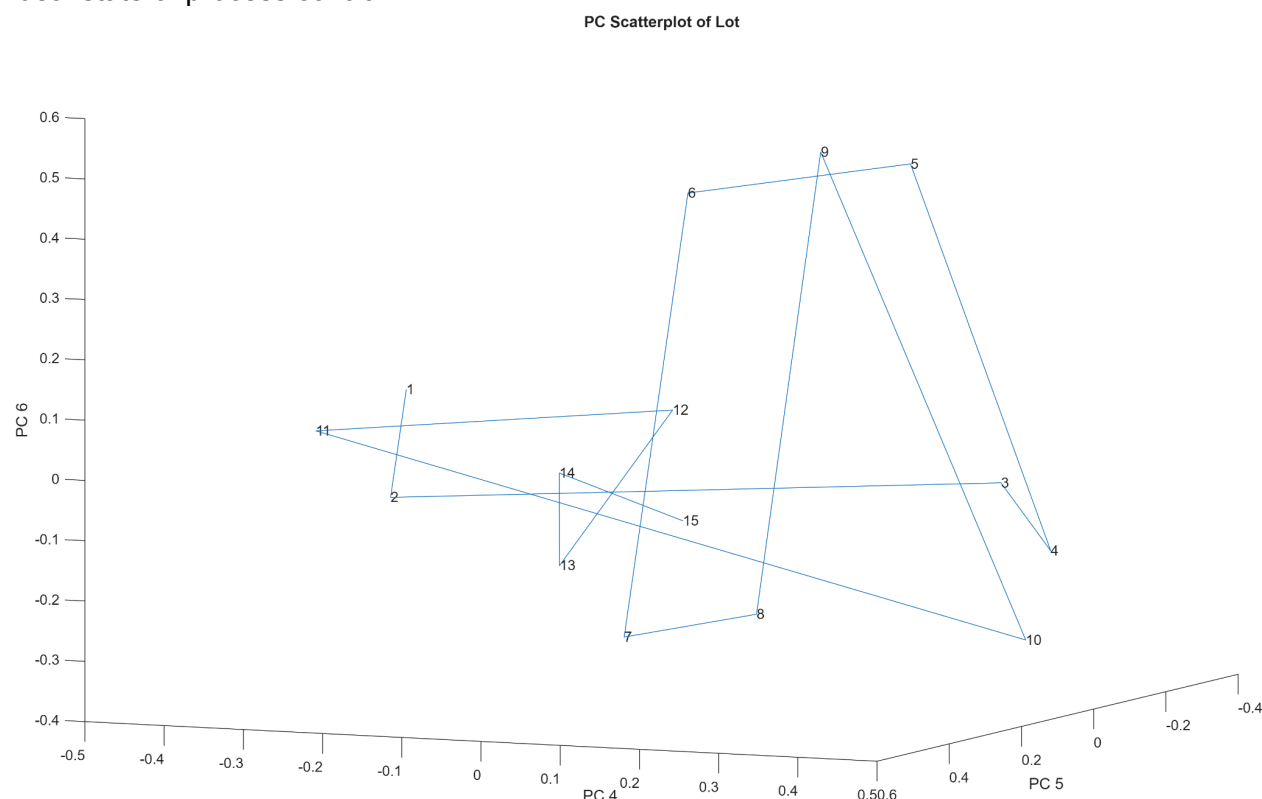


Figure 4. PC scatterplot of 15 vials from lot 2515203. The pattern in these PCs also suggests that there may be at least two groups in the data. In PCs 4 to 6, vials 5, 6, and 9 appear to be forming a second group.

[Figure 4](#) displays a PC scatterplot evaluating the higher-order principal components (specifically PCs 4 through 6) of the FTNIR spectra for the same 15 vials from lot 2515203 of Mycophenolate Mofetil for Injection. The importance of [Figure 4](#) lies in its ability to reveal subtle spectral variations that are masked by the dominant variance captured in the first few principal components. While PC 1 accounts for over 94% of total variation, inspecting PCs 4 to 6 allows for finer subcluster detection. In this higher-dimensional space, Vials 5, 6, and 9 appear to form a distinct secondary group. This reinforces the findings from [Figure 3](#) by providing further multidimensional evidence of intralot heterogeneity and subclustering within a single manufacturing batch.

[Figure 5](#) displays a PC scatterplot of the FTNIR spectra for the 15 vials from lot 2515203 of Mycophenolate Mofetil for Injection, evaluating higher-order principal components. Unlike [Figure 3](#) and [Figure 4](#), [Figure 5](#) does not exhibit obvious subclustering or distinct secondary groups, but it highlights vials 7 and 11 as potential individual outliers. [Figure 5](#) is important because it ensures a comprehensive, multidimensional evaluation across the principal components, capturing subtle, isolated anomalous behavior that does not organize into coherent subclusters. By demonstrating where the dataset transitions from structured subclustering to sporadic outlier variation, [Figure 5](#) completes the intralot principal component screening and reinforces the total extent of spectral variability present within a single batch.

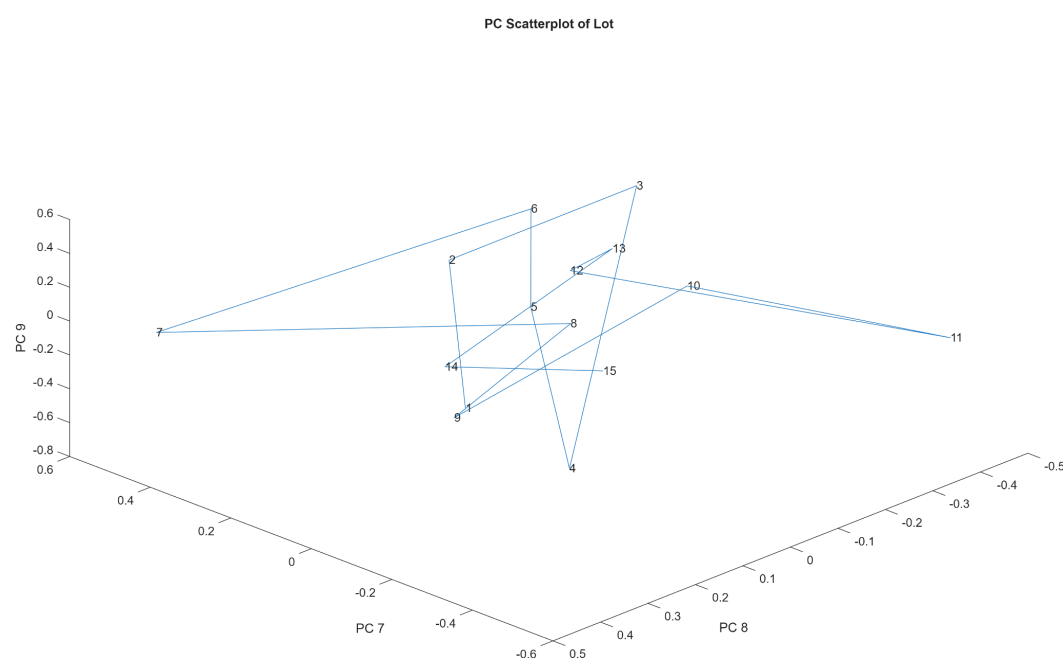


Figure 5. PC scatterplot of 15 vials from lot 2515203. There are no obvious groups in these PCs but vials 7 and 11 appear to be possible outliers.

[Table 1](#) displays the proportion of spectral variation accounted for by each of the first nine principal components derived from the FTNIR spectra of the 15 vials in Lot 2515203, alongside their cumulative PC variation. It shows that PC 1 accounts for the vast majority of total spectral variation at 94.60%, with subsequent components accounting for progressively smaller fractions, reaching a cumulative total of 99.97% through PC 9. Including [Table 1](#) is important because it provides the quantitative foundation for interpreting the principal component scatterplots, mathematically explaining how variance is distributed across the orthogonal dimensions. Furthermore, it contextualizes the analysis by noting that while PC 1 captures over 90% of the total variation, this proportion is likely higher than what would be observed across a larger sample size. This mathematical baseline helps justify examining higher-order PCs (such as PCs 4–6) to detect subtle intralot subclustering and potential outliers that might otherwise be obscured by the overwhelming dominance of PC 1.

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

PC Number	Variation in this PC	Cumulative PC Variation
1	0.9460	0.9460
2	0.0311	0.9771
3	0.0161	0.9932
4	0.0048	0.9980
5	0.0010	0.9990
6	0.0004	0.9994
7	0.0002	0.9995
8	0.0001	0.9997
9	0.0001	0.9997

Distance Measurement Results

The distance measurement results suggest that there is significant intra-lot heterogeneity and structural non-uniformity among vials within the exact same manufacturing batch (Lot 2515203). Vial 5 is 5.1 multidimensional standard deviations from the center of the other cluster, while vial 13 is 9.7 SDs away, vial 14 is 9.6 standard deviations away, and vial 15 is 11.6 standard deviations away from the center of the other vials. The difference in distances between vial 13 and 14, which seem close together, and to vial 15 is an artifact of the viewing angle. In near-infrared multivariate statistics, deviations of this magnitude (well beyond 3 SDs) indicate that these samples are not simply experiencing expected random measurement noise. Instead, they demonstrate that vials produced within a single lot possess distinct spectral variations. These variations can stem from physical or chemical inconsistencies—such as differences in sample packing, particle-size distributions, moisture absorption, or active ingredient/excipient concentrations. These results were sufficient to prompt an investigation of additional lots of the drug.

Interlot analysis

Smoothed spectra graph of library

[Figure 6](#) shows the smoothed FTNIR spectra of all 464 vials in the spectral library, collected non-invasively and non-destructively through the bottom of the vials across 18 distinct lots of Mycophenolate Mofetil for Injection. The figure transitions the study from evaluating a single, isolated lot (lot 2515203) to establishing a comprehensive baseline for inter-lot variability across a multiyear library of 18 different manufacturing lots. It displays the entire envelope of absorbance variability across the entire 464-vial sample set, serving as the foundational raw spectral dataset for all subsequent library-wide multivariate statistical analyses. The spectral

data compiled in [Figure 6](#) directly feeds into the principal component analyses allowing researchers to evaluate whether the intralot subclustering observed in initial testing persists across the broader manufacturing spectrum.

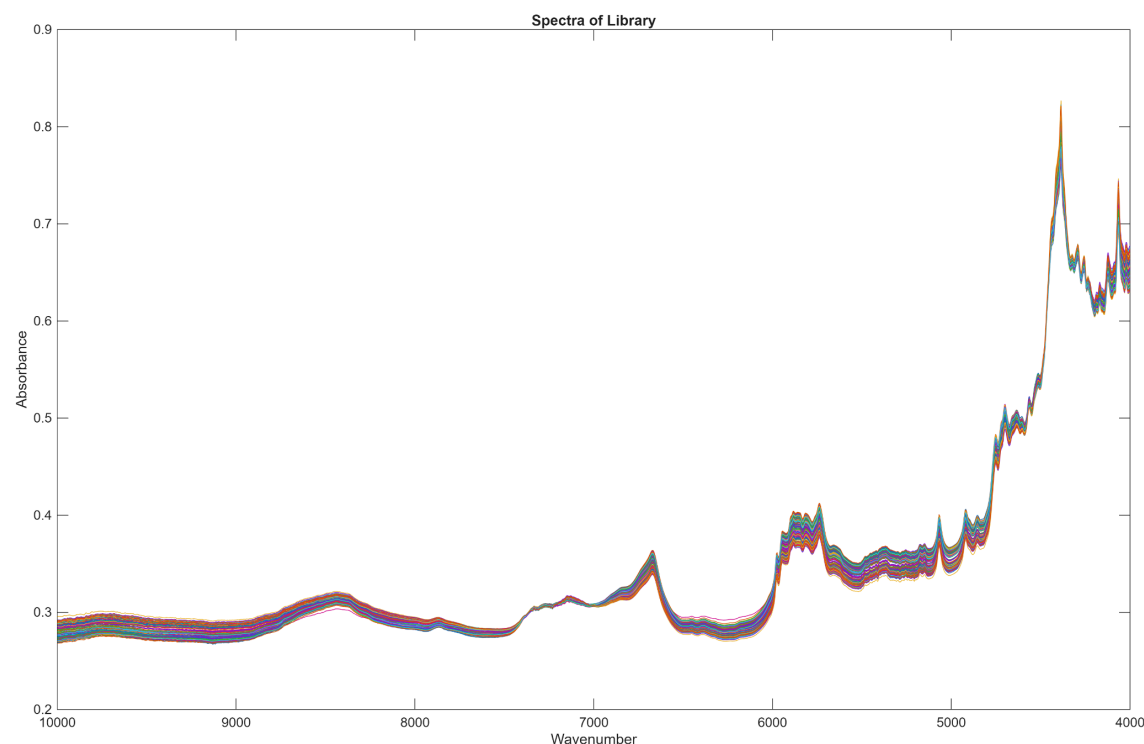


Figure 6. Spectra of all 464 vials in the spectral library of 18 lots of the drug product. The spectra were obtained noninvasively through the bottom of the vials.

PC Plots

[Figure 7](#) displays a Principal Component (PC) scatterplot of the Fourier transform near-infrared (FTNIR) spectra for all 464 vials across 18 distinct manufacturing lots of Mycophenolate Mofetil for Injection contained in the spectral library. The primary importance of [Figure 7](#) is that it confirms the subclustering behavior observed earlier in a single batch (lot 2515203 in [Figure 3](#)) is not an isolated, one-off anomaly. Instead, the scatterplot demonstrates that the entire 18-lot library segregates into two distinct spectral groups. By demonstrating that hundreds of vials spanning multiple production runs split into separate clusters in hyperspace, [Figure 7](#) provides strong empirical evidence of persistent, systematic variations in the drug product or manufacturing process over time. Consequently, it establishes the foundational multilot spatial distribution in principal component space and serves as the benchmark for evaluating higher-order principal components across the entire library.

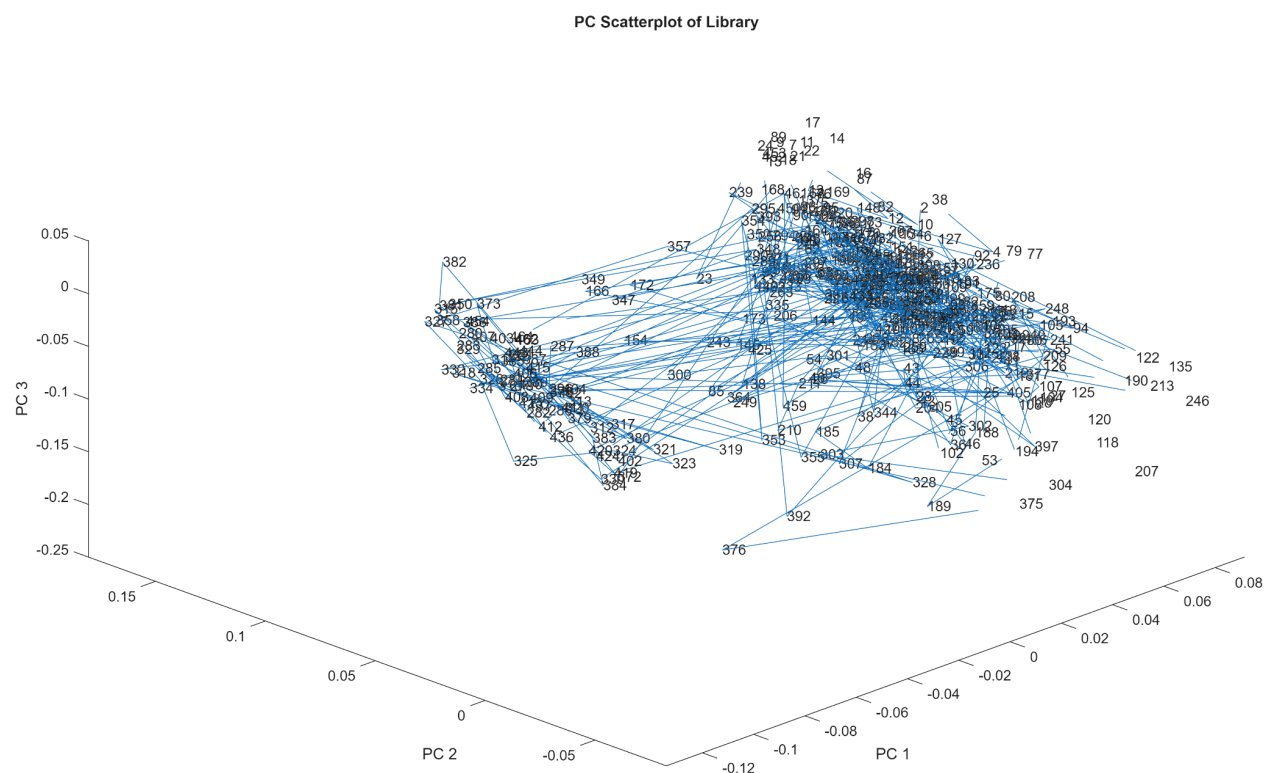
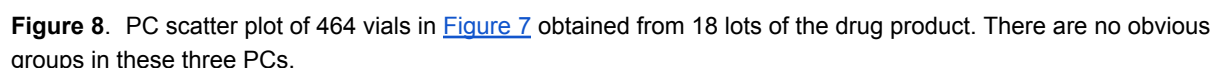


Figure 7. PC scatter plot of 464 vials in [Figure 6](#) obtained from 18 lots of the drug product. There appear to be two groups of spectra in these 18 lots, similar to what was observed in one lot in [Figure 2](#).

[Figure 8](#) displays a Principal Component (PC) scatterplot evaluating higher-order principal components for the entire 464-vial spectral library across 18 lots of Mycophenolate Mofetil for Injection. The primary importance of [Figure 8](#) lies in its demonstration that, unlike [Figure 7](#), which reveals two distinct clusters in lower-order principal components, these specific higher-order PCs show no obvious groups or secondary subclusters. Including [Figure 8](#) is important because it provides a complete, multidimensional profile of the multilot library, confirming that certain dimensions of spectral variance remain continuously and uniformly distributed without discrete batch or process separations. Ultimately, this helps differentiate between specific dimensions in spectral space that actively drive structural subclustering and those that merely represent background, uniform variance across the entire sample set.



In Principal Component Analysis, orthogonal axes are constructed sequentially to capture the maximum remaining variance in a multidimensional dataset. This means that different groups of principal components often isolate distinct physical, chemical, or instrumental factors. Principal components 1 through 3 account for the vast majority of overall variance, primarily capturing macro-level structural differences across the samples, such as primary active ingredient concentrations, major physical cake packing variations, or baseline absorbance shifts. Intermediate components (PCs 4 through 6) capture mid-level, continuous variance, such as uniform instrumental noise or smooth, continuous physical drift, which distributes evenly across the dataset without forming discrete spatial boundaries. Higher-order components (PCs 7 through 9), despite explaining a very small percentage of the total variance, isolate fine-grained, localized spectral variations. At this higher level of sensitivity, minor chemical perturbations, such as trace moisture absorption, low-level contaminants, subtle excipient state changes, or specific molecular vibrational shifts (as evidenced by distinct loading peaks plotted later) re-emerge as distinct subclusters. Thus, subclustering appears in PCs 1–3 due to macro-level structural variance, disappears in PCs 4–6 as continuous noise dominates, and reappears in PCs 7–9 as localized, fine-scale chemical or physical variations are isolated (see [Figure 9](#)).

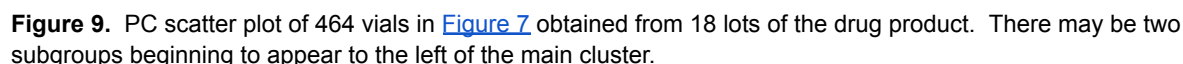


Table 2: Variation accounted for by each of the principal components of the spectra in the library

PC Number	Variation in this PC	Cumulative PC Variation
1	0.8782	0.8782
2	0.0715	0.9496
3	0.0212	0.9708
4	0.0156	0.9865
5	0.0050	0.9915
6	0.0038	0.9953
7	0.0013	0.9965
8	0.0008	0.9973

9	0.0005	0.9978
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[Table 2](#) displays the proportion of spectral variation explained by each of the first nine principal components derived from the FTNIR spectra of the full 464-vial library across 18 manufacturing lots of Mycophenolate Mofetil for Injection, along with their cumulative PC variation. It reveals that Principal Component 1 (PC 1) accounts for 87.82% of the total spectral variance, with PC 2 adding 7.15% and PC 3 contributing 2.12%, reaching a cumulative total of 99.78% through PC 9. [Table 2](#) is important because it mathematically demonstrates that PC 1 captures less than 90% of the total spectral variation across the multilot library, which serves as a quantitative indicator that distinct subgroups or subclusters are likely to exist within the overall dataset. When comparing [Table 2](#) to [Table 1](#), notable differences emerge between the single-lot and multi-lot variance distributions. In [Table 1](#) (analyzing a single 15-vial lot), PC 1 accounts for a much higher proportion of total variance at 94.60%. In contrast, PC 1 in [Table 2](#) drops to 87.82%, shifting a greater proportion of variance into higher-order principal components, for instance, PC 2 accounts for only 3.11% of variance in [Table 1](#) compared to 7.15% in [Table 2](#). This redistribution of variance across additional principal components in the multi-lot library reflects the broader range of physical and chemical variations present across 18 separate manufacturing lots compared to the relatively tight variance observed within a single batch.

PC Loadings Plots

[Figure 10](#) shows the PC 1 loadings spectrum derived from FTNIR spectrometry of the 464-vial spectral library across 18 manufacturing lots of Mycophenolate Mofetil for Injection. The spectrum maps out the specific spectral features driving the primary component of variance across the multi-lot library, highlighting key spectral variations at wavenumbers 4458, 7078, and 7261 cm^{-1} . The figure shows the residual effects of the MSC baseline correction. This figure is important because PC 1 accounts for over 87% of the total spectral variation within the library. By capturing less than 90% of the overall variance (a lower proportion than the 94.60% observed within the single lot) the PC 1 loadings spectrum serves as a strong quantitative indicator that distinct subgroups and subclusters exist across the 18 production runs. Furthermore, identifying the specific vibrational absorption peaks at 4458, 7078, and 7261 cm^{-1} pinpoints the key chemical or physical factors, such as shifts in active pharmaceutical ingredient concentration, moisture absorption, or differences in the physical state of the lyophilized cake like particle size, that drive spectral divergence and suggest potential departures from ideal process control across drug lots.

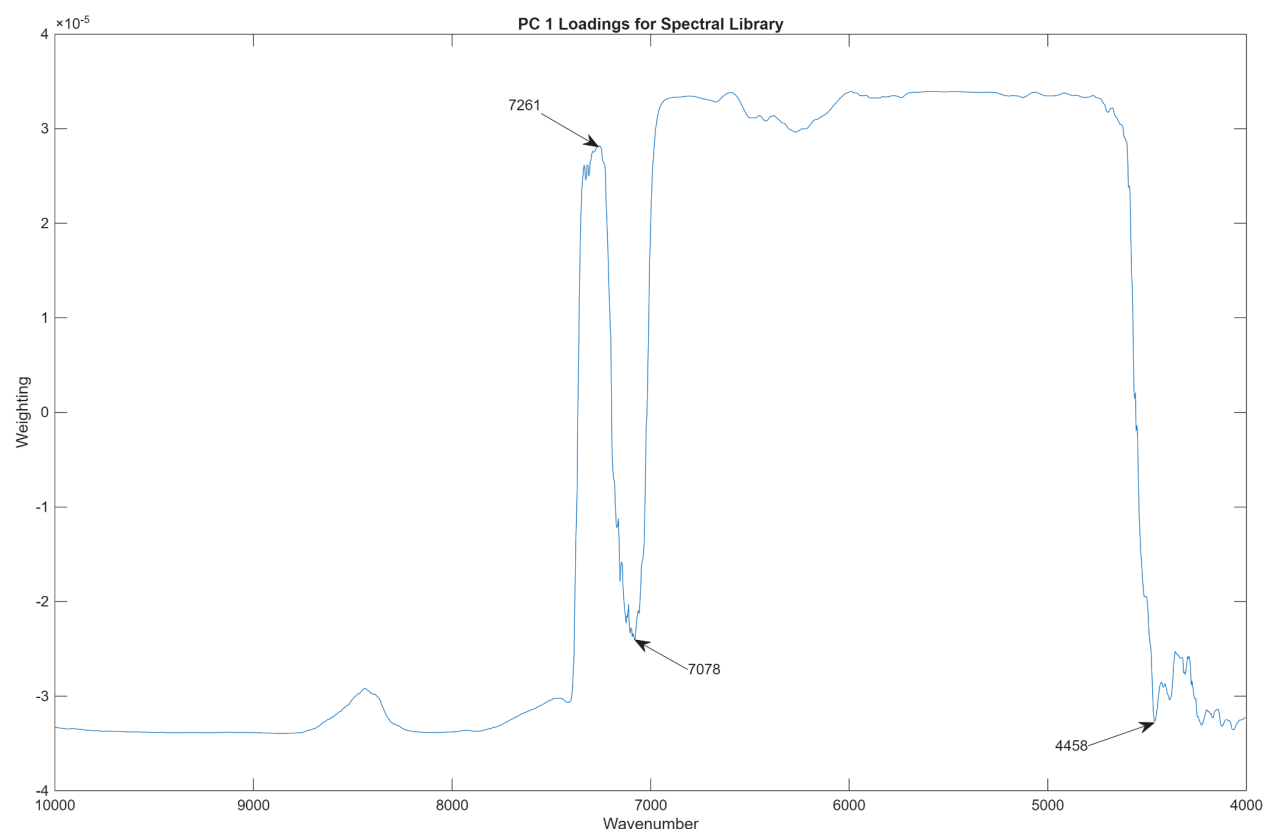


Figure 10. The PC 1 loadings spectrum of the spectral library of 464 vials. PC 1 captures over 87% of the total variation, suggesting that there are some subgroups in the data. Notable spectral variations are marked at 4458, 7078, and 7261 cm^{-1} .

[Figure 11](#) shows the PC 2 loadings spectrum generated from FTNIR spectrometry of the 464-vial spectral library across 18 manufacturing lots of Mycophenolate Mofetil for Injection. The plot illustrates the distribution of loading weights across the near-infrared spectrum, highlighting notable spectral variations at wavenumbers 4275, 4547, 6262, 7189, 7472, and 8443 cm^{-1} .

This figure is important because PC 2 accounts for 7.15% of the total spectral variation across the multi-lot library, representing the second largest orthogonal dimension of variance in the dataset. While PC 1 accounts for 87.82% of the variance, PC 2 captures secondary chemical and physical variations that are masked by the dominant first component. Examining the PC 2 loadings spectrum is critical for identifying specific vibrational band shifts and spectral regions (marked by key absorption peaks at 4275, 4547, 6262, 7189, 7472, and 8443 cm^{-1}) that contribute to the multi-dimensional dispersion and subclustering of drug vials in principal component hyperspace, further illustrating potential inter-lot inconsistencies and departures from uniform manufacturing process control.

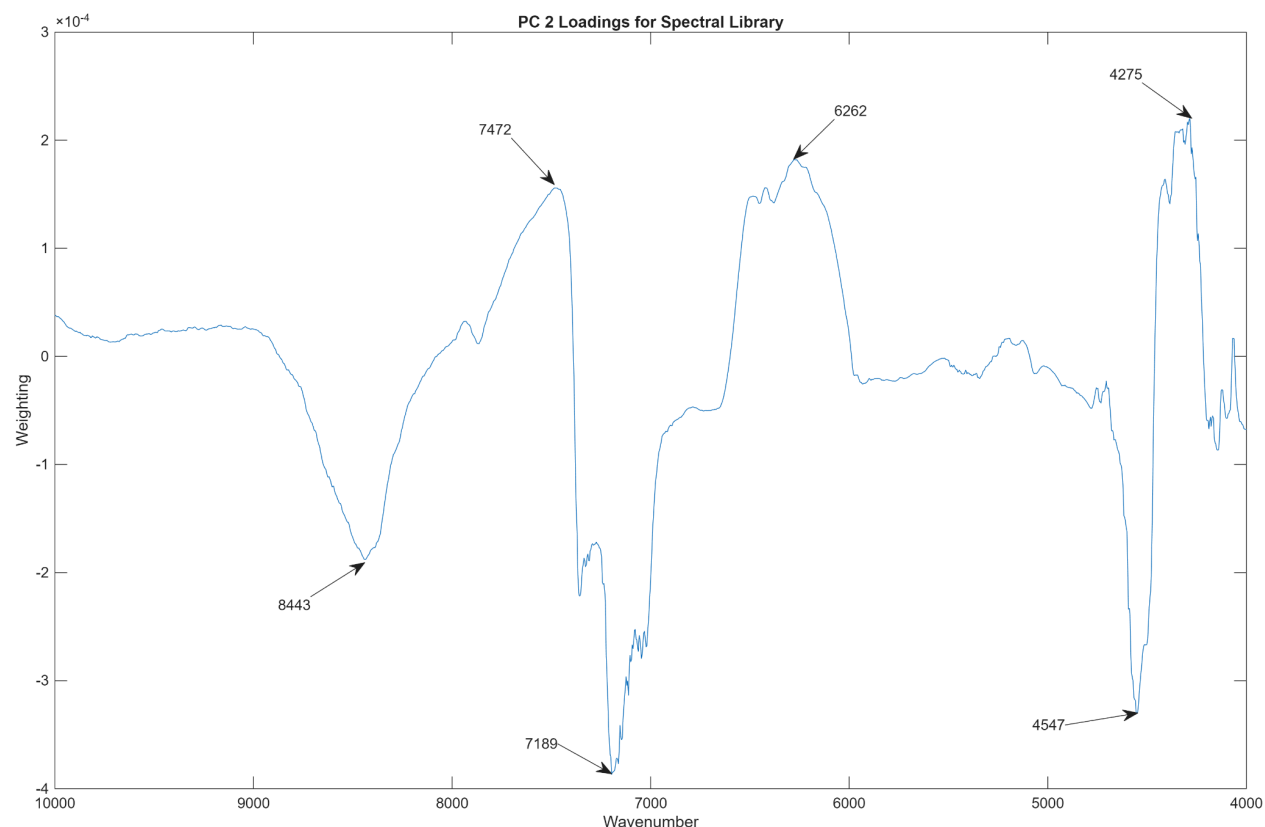


Figure 11. The PC 2 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4275, 4547, 6262, 7189, 7472, and 8443 cm^{-1} .

In a Principal Component Analysis loadings spectrum like [Figure 11](#), the distinction between positive and negative peaks represents the relative direction and correlation of spectral absorbance changes across the sample set along that specific principal component axis. Positive peaks indicate spectral wavenumbers where an increase in infrared absorbance correlates positively with higher scores on PC 2. Conversely, negative peaks indicate wavenumbers where higher PC 2 scores correspond to a relative decrease or suppression in absorbance.

This opposing orientation highlights inverse relationships between specific chemical or physical variables across the 464 vials in the library. For instance, a positive peak at one wavenumber paired with a negative peak at another often reflects complementary vibrational state shifts, such as a trade-off between moisture absorption and active ingredient decomposition. Ultimately, the mixture of positive and negative loadings at key frequencies, such as 4275, 4547, 6262, 7189, 7472, and 8443 cm^{-1} , shows that PC 2 is measuring a dynamic, multifactor variance where certain spectral features rise as others fall, driving the spatial segregation and subclustering of different manufacturing lots in multidimensional hyperspace.

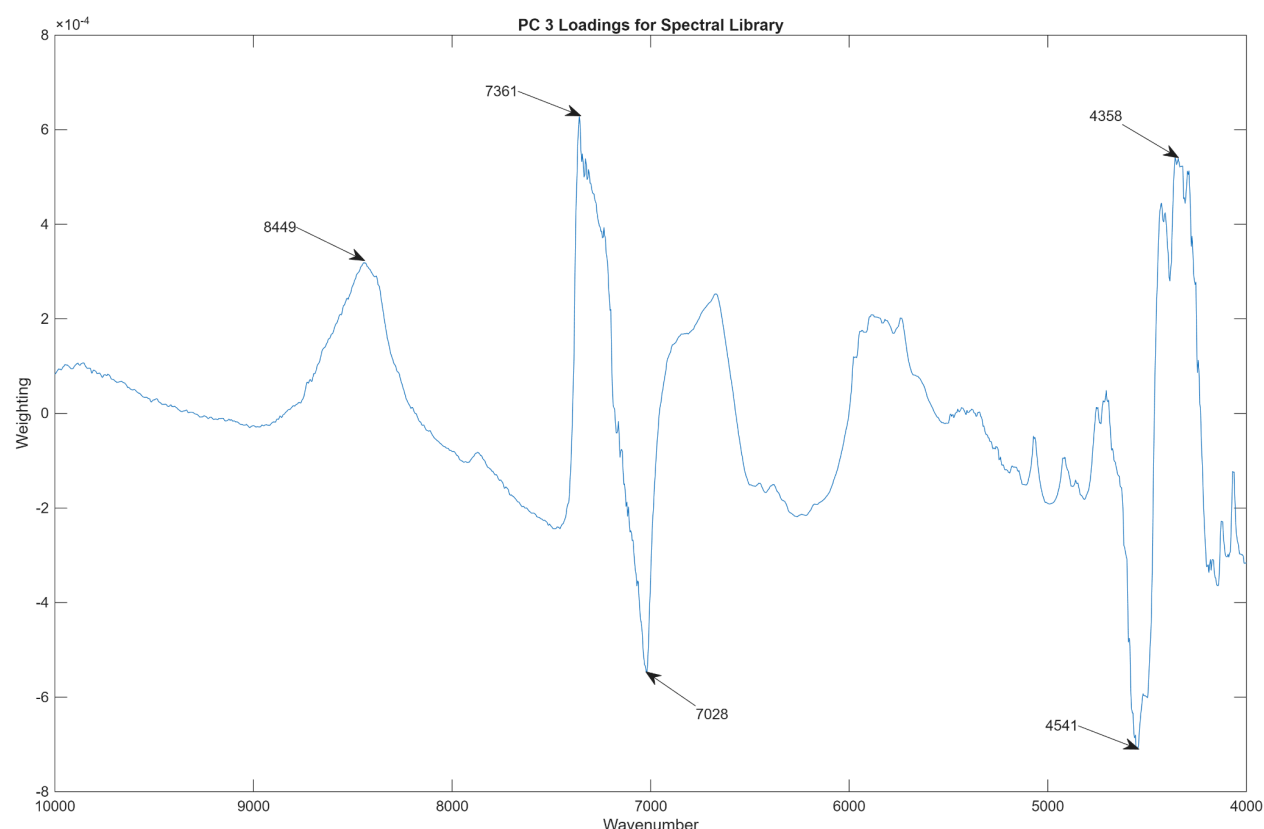


Figure 12. The PC 3 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4358, 4541, 7028, 7361, and 8449 cm^{-1} .

[Figure 12](#) shows the PC 3 loadings spectrum generated from FTNIR spectrometry across the 464-vial spectral library representing 18 manufacturing lots of Mycophenolate Mofetil for Injection. This figure is important because PC 3 isolates the third orthogonal dimension of spectral variance, accounting for 2.12% of the overall variation across the multi-lot library. Mapping loading weights across the wavenumber spectrum highlights key spectral regions that contribute to the multidimensional dispersion of drug samples, revealing subtle inter-lot chemical and physical inconsistencies that suggest potential departures from uniform manufacturing process control. The prominent loadings peaks marked in [Figure 12](#) correspond to fundamental vibrational modes operating in the near-infrared overtone and combination regions. The peaks at lower wavenumbers, specifically 4358 and 4541 cm^{-1} , fall within the combination band region (4000–5000 cm^{-1}), where absorptions typically arise from combined C–H stretching and C–H bending/deformation modes in the ester and morpholino groups of mycophenolate mofetil, as well as N–H or O–H stretch-bend combinations. Moving to higher wavenumbers, the absorption features at 7028 and 7361 cm^{-1} reside in the first overtone region (6800–7500 cm^{-1}), which generally corresponds to the first overtone of O–H stretching ($2\nu_{\text{O-H}}$) associated with trace

moisture absorption or the first overtone of N–H stretching ($2\nu_{\text{N-H}}$) in the tertiary amine or amide environments. Finally, the peak at 8449 cm^{-1} is located in the second overtone region (ca. $8000\text{--}8800\text{ cm}^{-1}$), corresponding predominantly to the second overtones of C–H stretching vibrations ($3\nu_{\text{C-H}}$) in aliphatic methyl, methylene, or aromatic ring systems.

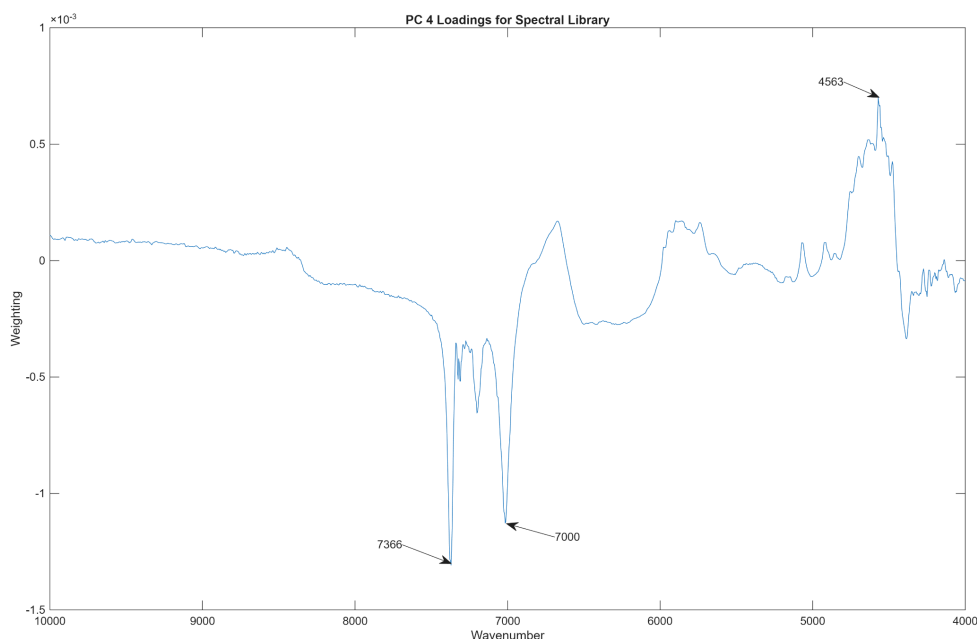


Figure 13. The PC 4 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4563 , 7000 , and 7366 cm^{-1} .

[Figure 13](#) shows the PC 4 loadings spectrum generated from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 4 isolates the fourth orthogonal dimension of variance, accounting for 1.56% of total spectral variation across the 18 manufacturing lots. By plotting loading weights across the wavenumber spectrum, it identifies mid-level, continuous spectral variations and instrumental noise features, helping to establish how background variance is distributed and differentiating continuous noise from the localized chemical or physical drivers of lot dispersion.

The notable loading peaks marked in [Figure 13](#) at 4563 , 7000 , and 7366 cm^{-1} correspond to fundamental vibrational modes operating within the near-infrared combination and first overtone regions. The peak at 4563 cm^{-1} resides in the combination region (ca. $4000\text{--}5000\text{ cm}^{-1}$, where NIR absorption typically arises from combination bands of C–H stretching combined with C–H bending/deformation vibrations in the morpholino ring and aliphatic chains of mycophenolate mofetil, or potential O–H/N–H stretch-bend combinations. Moving to higher wavenumbers, the prominent peak at 7000 cm^{-1} lies in the first overtone region (ca. $6800\text{--}7100\text{ cm}^{-1}$) and generally corresponds to the first overtone of O–H stretching ($2\nu_{\text{O-H}}$) associated with trace moisture absorption or free/bound hydroxyl groups, as well as N–H stretching overtones ($2\nu_{\text{N-H}}$). Finally,

the peak at 7366 cm^{-1} falls within the upper first overtone region (ca. $7100\text{--}7500\text{ cm}^{-1}$), corresponding primarily to the first overtones of C–H stretching vibrations ($2\nu_{\text{C-H}}$) in aromatic or aliphatic groups, as well as higher-order C–H combination modes in the active drug molecule.

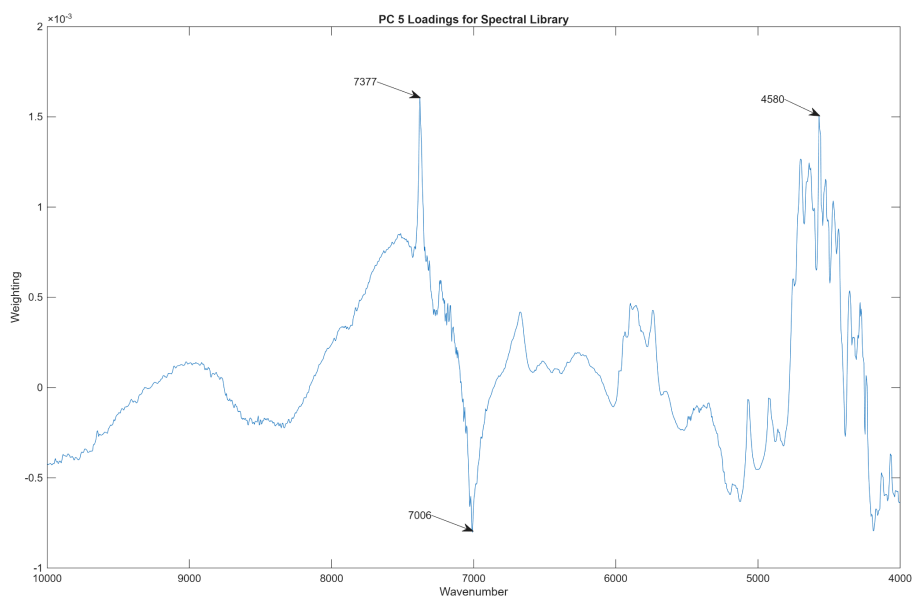


Figure 14. The PC 5 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4580 , 7006 , and 7377 cm^{-1} .

[Figure 14](#) shows the PC 5 loadings spectrum derived from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 5 captures the fifth orthogonal dimension of variance, accounting for 0.50% of the total spectral variation across the 18 manufacturing lots. By plotting loading weights across the wavenumber spectrum, it highlights localized, mid-to-higher order spectral variations that help characterize secondary physical and chemical variations across drug lots, distinguishing fine spectral details from the dominant variance captured in earlier principal components.

The specific loading peaks marked in [Figure 14](#) at 4580 , 7006 , and 7377 cm^{-1} correspond to fundamental molecular vibrations in the combination and first overtone regions of the near-infrared spectrum. The absorption feature at 4580 cm^{-1} falls in the combination band region (ca. $4000\text{--}5000\text{ cm}^{-1}$, where peaks typically arise from combination modes involving C–H stretching and C–H bending or deformation within the aliphatic chains and morpholino ring of mycophenolate mofetil, as well as N–H or O–H stretch-bend combinations. Higher in the spectrum, the peak at 7006 cm^{-1} resides in the first overtone region ($6800\text{--}7100\text{ cm}^{-1}$), which generally corresponds to the first overtone of O–H stretching vibrations ($2\nu_{\text{O-H}}$) from trace moisture or hydrogen-bonded hydroxyl groups, along with potential first overtones of N–H stretching ($2\nu_{\text{N-H}}$). Lastly, the feature at 7377 cm^{-1} lies in the upper first overtone region (ca.

7100-7500 cm^{-1} , corresponding primarily to the first overtones of aliphatic and aromatic C–H stretching vibrations ($2\nu_{\text{C-H}}$) as well as complex C–H combination bands.

In near-infrared spectrometry, raw absorbance spectra are dominated by broad, heavily overlapping absorption envelopes caused by anharmonicity, overlapping overtone and combination bands, and baseline light-scattering variations. Loadings spectra in Principal Component Analysis frequently display noticeably sharper, more distinct peaks than the underlying absorbance spectra due to the mathematical nature of variance decomposition and spectral resolution enhancement.

The primary reason for this visual sharpening is that PCA loadings function similarly to mathematical derivative filters or contrast/difference spectra. Rather than measuring total light absorption at a given wavenumber, a loading weight reflects the degree to which absorbance at that specific wavenumber varies across the sample set independently of other wavelengths. When a broad absorption band undergoes slight frequency shifts, intensity changes, or bandwidth alterations across different samples (such as from variations in moisture content, physical particle packing, or active ingredient concentration) the point-by-point variance peaks sharply at the inflection points and slopes of the band. This produces sharp positive and negative features (often resembling derivative line shapes) in the loadings plot, even when the original absorbance spectrum displays only a broad, featureless hump.

Additionally, baseline correction techniques (such as Multiplicative Scatter Correction) and the orthogonal structure of PCA separate broad, continuous background variations (like physical scattering or macro-level pathlength differences) into lower-order components. By isolating broad physical scatter from subtle, localized chemical absorption changes, higher-order principal components subtract out the underlying background continuum. This mathematical isolation deconvolves complex, overlapping spectral bands, resolving distinct vibrational features that are otherwise obscured beneath the broad absorption envelopes of the raw spectra.

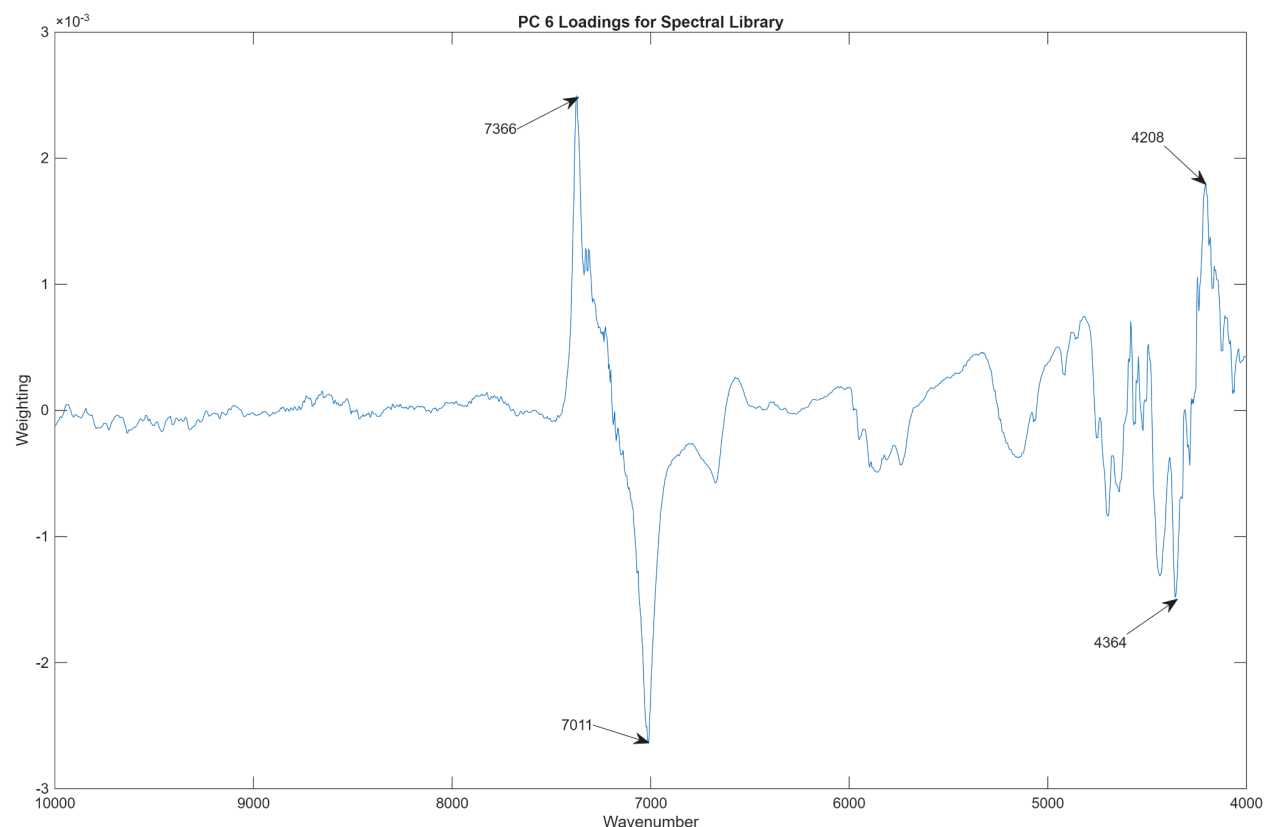


Figure 15. The PC 6 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4208, 4364, 7011, and 7366 cm^{-1}

[Figure 15](#) shows the PC 6 loadings spectrum derived from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 6 isolates the sixth orthogonal dimension of variance, capturing fine-grained, localized spectral variations across the 18 manufacturing lots. Mapping loading weights across the wavenumber spectrum highlights specific vibrational band shifts that contribute to the multidimensional dispersion of drug samples, revealing subtle interlot chemical and physical inconsistencies that suggest potential departures from uniform manufacturing process control.

The prominent loadings peaks marked in [Figure 15](#) at 4208, 4364, 7011, and 7366 cm^{-1} correspond to fundamental vibrational modes operating within the near-infrared combination and first overtone regions. The peaks at lower wavenumbers, specifically 4208 and 4364 cm^{-1} , fall within the combination band region (4000-5000 cm^{-1}), where absorptions typically arise from combined C–H stretching and C–H bending/deformation modes within the morpholino ring and aliphatic chains of mycophenolate mofetil, as well as N–H or O–H stretch-bend combinations. Moving to higher wavenumbers, the sharp negative feature at 7011 cm^{-1} resides in the first

overtone region ($6800\text{--}7100\text{ cm}^{-1}$), corresponding primarily to the first overtone of O–H stretching ($2\nu_{\text{O-H}}$) associated with trace moisture absorption or free/bound hydroxyl groups, alongside potential N–H stretching overtones ($2\nu_{\text{N-H}}$). Lastly, the strong positive peak at 7366 cm^{-1} lies in the upper first overtone region ($7100\text{--}7500\text{ cm}^{-1}$), corresponding predominantly to the first overtones of aliphatic and aromatic C–H stretching vibrations ($2\nu_{\text{C-H}}$) as well as higher-order C–H combination modes in the active drug molecule.

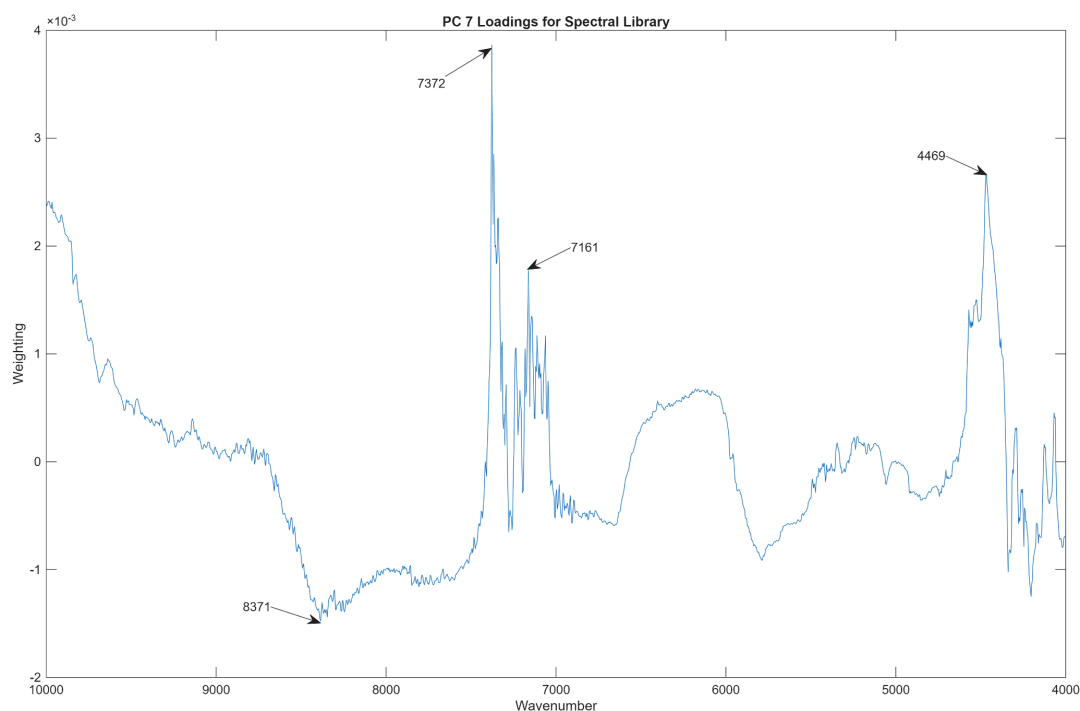


Figure 16. The PC 7 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4469 , 7161 , 7372 , and 8371 cm^{-1} .

[Figure 16](#) shows the PC 7 loadings spectrum generated from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 7 isolates the seventh orthogonal dimension of spectral variance, which captures high-order, fine-grained physical and chemical variations across the 18 manufacturing lots. By mapping loading weights across the wavenumber spectrum, it helps identify subtle vibrational band shifts and localized spectral features that contribute to sample dispersion in higher-dimensional hyperspace, providing insight into fine-scale inter-lot inconsistencies and departures from uniform process control.

The marked loadings peaks in [Figure 16](#) at 4469 , 7161 , 7372 , and 8371 cm^{-1} correspond to fundamental molecular vibrations spanning the combination, first overtone, and second overtone regions of the near-infrared spectrum. The peak at 4469 cm^{-1} falls within the combination band region $4000\text{--}5000\text{ cm}^{-1}$, where absorptions typically arise from C–H stretching

combined with C–H bending/deformation modes in the morpholino ring and aliphatic structures of mycophenolate mofetil, as well as N–H or O–H stretch-bend combinations. Moving to higher wavenumbers, the sharp features at 7161 and 7372 cm^{-1} lie in the first overtone region (6800–7500 cm^{-1}), corresponding to the first overtones of O–H stretching ($2\nu_{\text{O-H}}$) from trace moisture or hydroxyl environments, N–H stretching overtones ($2\nu_{\text{N-H}}$), and first overtones of aliphatic or aromatic C–H stretching vibrations ($2\nu_{\text{C-H}}$). Finally, the negative feature at 8371 cm^{-1} resides in the second overtone region (8000–8800 cm^{-1}), which corresponds predominantly to the second overtones of C–H stretching vibrations ($2\nu_{\text{C-H}}$) in methyl, methylene, or aromatic ring systems.

[Figure 17](#) shows the PC 8 loadings spectrum derived from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 8 isolates a higher-order, fine-grained dimension of spectral variance, accounting for 0.08% of the total spectral variation across the 18 manufacturing lots. At this higher level of sensitivity, lower-order background noise is filtered out, allowing PC 8 to re-isolate localized, subtle chemical perturbations and specific molecular vibrational shifts that contribute to subclustering and small-scale inter-lot variations across production runs.

The marked loadings peaks in [Figure 17](#) at 4702, 7006, 7161, and 7377 cm^{-1} correspond to distinct vibrational modes in the combination and first overtone regions of the near-infrared spectrum. The peak at 4702 cm^{-1} lies in the combination region (4000–5000 cm^{-1} , where NIR absorbances typically stem from combination bands of C–H stretching and C–H deformation/bending vibrations in aromatic or aliphatic groups, alongside potential N–H or O–H combination modes. The peaks at 7006 and 7161 cm^{-1} reside in the lower first overtone region (6800–7200 cm^{-1} , corresponding primarily to the first overtone of O–H stretching ($2\nu_{\text{O-H}}$) from trace moisture or hydrogen-bonded hydroxyl species, as well as the first overtone of N–H stretching ($2\nu_{\text{N-H}}$) in amine or amide environments. Finally, the peak at 7377 cm^{-1} falls in the upper first overtone region (7200–7500 cm^{-1}), corresponding mainly to the first overtones of aliphatic and aromatic C–H stretching vibrations ($2\nu_{\text{C-H}}$) in the drug molecule.

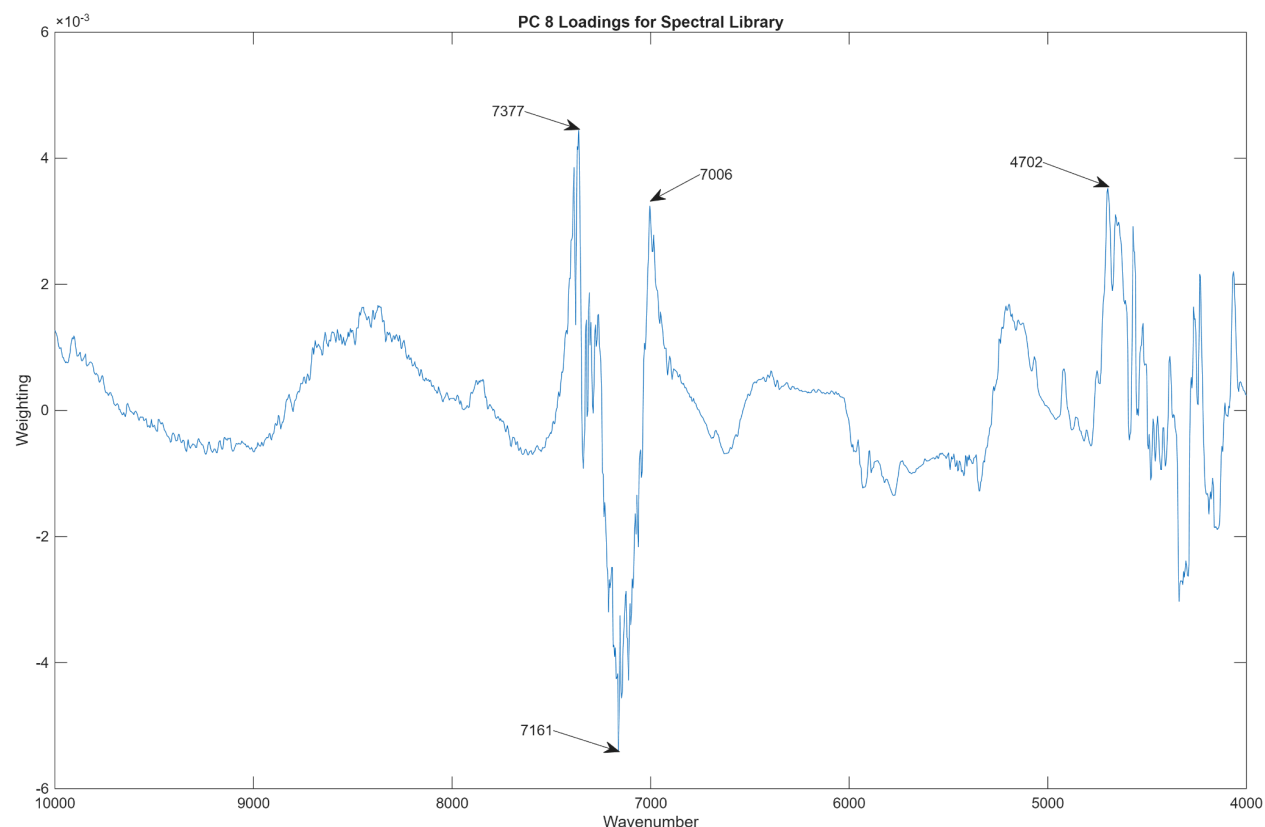


Figure 17. The PC 8 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4702, 7006, 7161, and 7377 cm^{-1} .

[Figure 18](#) shows the PC 9 loadings spectrum generated from FTNIR spectrometry across the 464-vial spectral library of Mycophenolate Mofetil for Injection. This figure is important because PC 9 captures a high-order, fine-scale dimension of spectral variance, accounting for 0.05% of the total variation across the 18 manufacturing lots. Noise is beginning to enter the spectrum from the high wave number end of the spectrum. While accounting for a very small fraction of the overall variance, PC 9 filters out macro-level background noise to isolate localized, subtle chemical or physical variations, such as minor excipient state changes, trace moisture uptake, or specific molecular vibrational shifts, that contribute to the fine structural subclustering of drug vials in principal component space. The marked loadings peaks in [Figure 18](#) at 4469, 5135, and 7372 cm^{-1} correspond to specific molecular vibrations operating across the combination and first overtone regions of the near-infrared spectrum. The absorption feature at 4469 cm^{-1} falls within the combination region (4000-5000 cm^{-1}), where NIR signals typically arise from combination modes of C–H stretching paired with C–H bending/deformation in the morpholino ring and aliphatic chains of the active molecule, as well as possible N–H or O–H stretch-bend combinations. The peak at 5135 cm^{-1} lies in the water combination band region (5100-5300 cm^{-1} , which corresponds directly to the combination of O–H asymmetric stretching and O–H bending

($\nu_{\text{O-H}} + \delta_{\text{O-H}}$) characteristic of molecular water or hydration states. Finally, the peak at 7372 cm^{-1} resides in the upper first overtone region ($7100\text{--}7500\text{ cm}^{-1}$), corresponding predominantly to the first overtone of C–H stretching vibrations ($2\nu_{\text{C-H}}$) in aromatic and aliphatic environments.

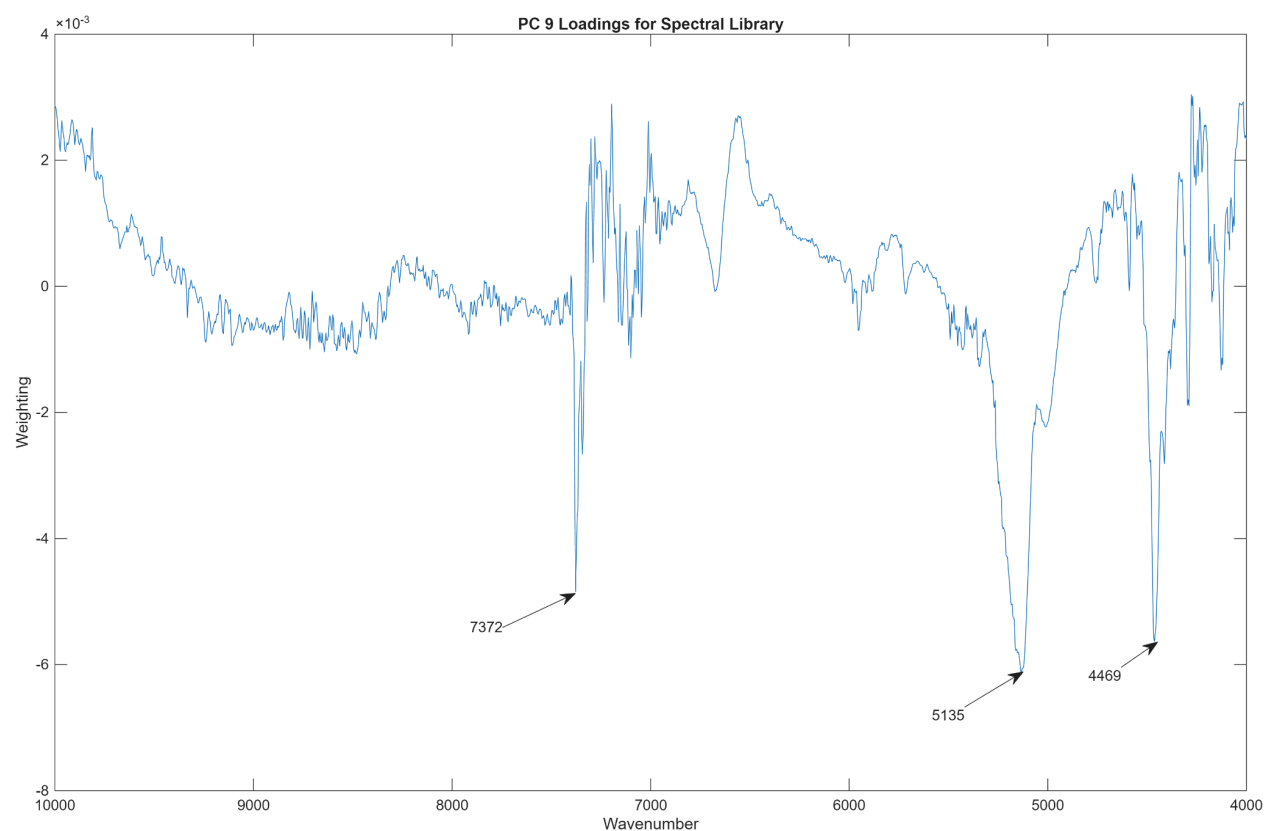


Figure 18. The PC 9 loadings spectrum of the spectral library of 464 vials. Important spectral variations are marked at 4469 , 5135 , and 7372 cm^{-1} . Noise is beginning to enter the spectrum from the high wave number end of the spectrum on the left.

In FTNIR spectrometry, noise levels often increase significantly at the high end of the wavenumber region (typically above $8000\text{--}10,000\text{ cm}^{-1}$, corresponding to short wavelengths below $1000\text{--}1200\text{ nm}$). This elevated noise is primarily driven by three interrelated physical and optical factors:

1. *Decreasing Source Emittance (Blackbody Radiation Curve):* FTNIR instruments typically rely on halogen or tungsten-filament lamps, which emit light following Planck's blackbody radiation law. The spectral energy distribution of these sources peaks in the low-to-mid NIR region ($4000\text{--}7000\text{ cm}^{-1}$) and falls off sharply toward higher wavenumbers (shorter

wavelengths entering the visible region). As photon flux from the source drops, the light reaching the detector decreases, reducing the overall signal-to-noise ratio.

2. *Detector Sensitivity Roll-Off:* Extended range Indium Gallium Arsenide (InGaAs) or Lead Sulfide (PbS) detectors commonly used in NIR spectrometers are optimized for lower energy photons ($4000\text{--}8000\text{ cm}^{-1}$). Near the bandgap edge at the high-wavenumber boundary, quantum efficiency and responsivity decrease. When optical signal strength diminishes relative to intrinsic electronic dark current and thermal detector noise, baseline noise increases.
3. *Increased Optical Rayleigh Scattering:* Light scattering by physical sample matrices (such as lyophilized drug cakes, powders, or suspensions) scales inversely with the fourth power of wavelength. Higher wavenumbers (shorter wavelengths) experience much stronger light scattering, which attenuates the directional unscattered beam returning to the detector and introduces multiplicative phase and pathlength fluctuations.

Subcluster Detection

[Figure 19](#) shows a quantile-quantile (QQ) plot generated by the FSOB subcluster detection algorithm, which evaluates probability-density contours in spectral hyperspace. The plot displays two distinct lines or trends rather than a single unified progression, corresponding to the separation of the 464-vial spectral library into a small cluster of 66 vials (14.2%) and a larger cluster of 398 vials (85.8%). This plot is important because it provides rigorous, nonparametric statistical confirmation that the observed spectral separation is genuine rather than an artifact of random measurement noise. The algorithm confirms a statistically significant difference between the two subclusters ($r_{\text{in}}=0.97$, $r_{\text{ts}}=0.83$, $p=0.02$). By demonstrating significant subclustering across multiple lots in the library, the figure offers empirical evidence that the manufacturing process may have operated outside an ideal state of uniform process control.

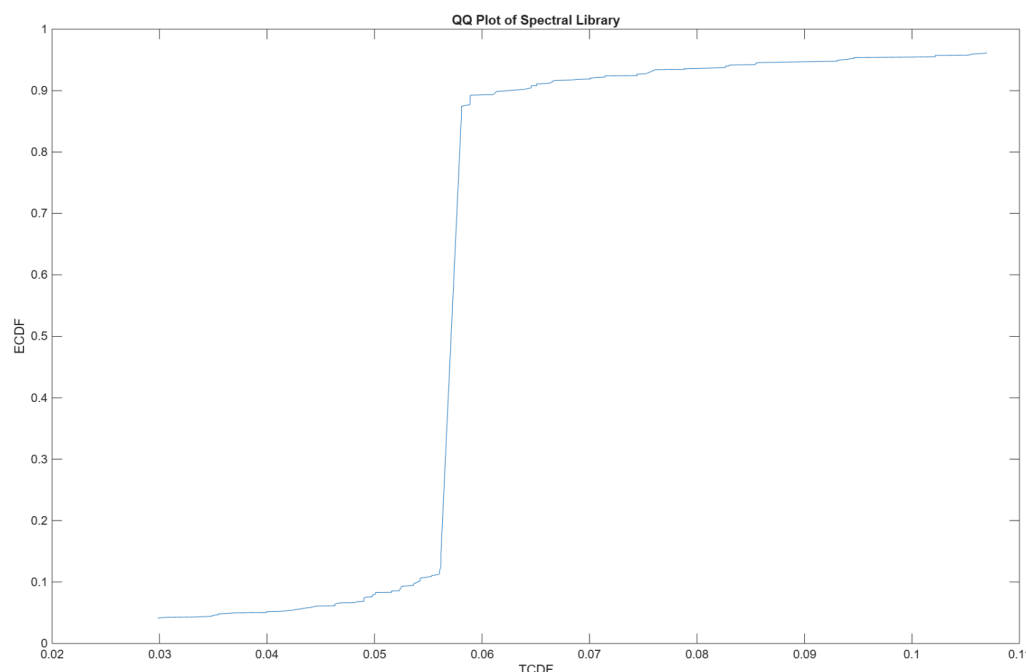


Figure 19. QQ plot from the subcluster detection algorithm shows two groups in the data ($r_{tn}=0.97$, $r_{ts}=0.83$, $p=0.02$).

Conclusion

Mycophenolate Mofetil for Injection is an antimetabolite immunosuppressant supplied as a sterile, lyophilized powder containing 500 mg of active drug per single-dose vial for intravenous administration. As a prodrug of mycophenolic acid (MPA), it selectively inhibits inosine monophosphate dehydrogenase (IMPDH) to block de novo guanosine nucleotide synthesis and inhibit T- and B-lymphocyte proliferation. Indicated in combination regimens for the prophylaxis of organ rejection in adult and pediatric allogeneic kidney, heart, or liver transplant recipients, the drug carries significant boxed warnings for embryofetal toxicity, serious opportunistic infections, and malignancies. Common adverse reactions include diarrhea, leukopenia, infection, vomiting, hypertension, and peripheral edema. Given its critical role and narrow therapeutic margin, non-destructive quality surveillance of its physical and chemical integrity remains essential.

Nondestructive FTNIR spectrometry coupled with multivariate statistical modeling revealed distinct patterns of intralot and interlot variability across the evaluated drug product. Initial screening of a single 15-vial batch (Lot 2515203) demonstrated significant intra-lot heterogeneity, where individual vials deviated up to 11.6 multidimensional standard deviations

from the main cluster center. Distance metrics derived from the Bootstrap Error-Adjusted Single-sample Technique identified multiple statistical outliers (including Vials 5, 13, 14, and 15) well beyond the ordinary 3 SD threshold in full spectral space as well as in principal component (PC) space. Higher-order PC projections (PCs 4–6) further revealed distinct secondary subclustering within this single manufacturing batch. Expanding the investigation to a multiyear spectral library comprising 464 vials across 18 distinct manufacturing lots confirmed that these spectral departures were not isolated occurrences. In the multilot library, Principal Component 1 accounted for 87.82% of the total spectral variance (falling below the 90% threshold typically observed in uniform single-lot systems) and the entire sample set segregated into two distinct spatial clusters in PC hyperspace. FSOB nonparametric density estimation in both full spectral space and PC space formally confirmed significant subclustering within the 464-vial library, splitting the library into a minor cluster of 66 vials (14.2%) and a major cluster of 398 vials (85.8%), supported by quantile-quantile (QQ) plot analysis ($r_{tn}=0.97$, $r_{ts}=0.83$, $p=0.02$).

These spectrometric results do not prove an excess level of impurities or active ingredient adulteration. However, they demonstrate that subtle physical or chemical variations (such as differences in sample packing, particle-size distribution, lyophilized cake structure, moisture uptake, or component concentrations) exist both within individual lots and across production runs. Ultimately, these findings suggest that the manufacturing process may have been operating outside an ideal state of statistical process control, highlighting the necessity of expanded process analytical technology (PAT) monitoring and additional analytical investigation. Additional investigation is needed.

Acknowledgements

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