

NIR Spectrometry Reveals Lack of Content Uniformity in Methylprednisolone Sodium Succinate

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RAPID COMMUNICATION

Abstract

Methylprednisolone sodium succinate for injection, USP is an anti-inflammatory glucocorticoid. The active ingredient is methylprednisolone sodium succinate, which is the sodium succinate ester of methylprednisolone. The substance is a white or nearly white, odorless, hygroscopic, amorphous solid. It is highly soluble in water and alcohol, insoluble in chloroform, and only slightly soluble in acetone. This injectable form of the drug is indicated for the treatment of various conditions, including some allergic states, dermatologic, endocrine, gastrointestinal, hematologic, neoplastic, nervous system, ophthalmic, renal, respiratory, and rheumatic disorders.

The analysis of a single lot of methylprednisolone sodium succinate revealed significant intra-lot variability in the near-infrared spectra. Differences were visually apparent in the absorbance peaks, particularly in the ranges of 4500 to 5000 cm^{-1} and 6000 to 6500 cm^{-1} . Further examination of a zoomed-in region (7000 to 8500 cm^{-1}) showed two spectra that were statistically significant outliers, positioned at 4.4 and 5.9 standard deviations (SDs) from the center of the sample cluster.

An analysis of a spectral library containing 242 vials of methylprednisolone sodium succinate from 26 different lots found significant interlot variability and statistically confirmed the presence of distinct subclusters. To statistically validate the visual findings, a subcluster detection algorithm was applied to the spectral data. The algorithm confirmed that two distinct groups exist within the spectral data. The measured correlation value ($r_{ts}=0.8704$) was below the 98% confidence level threshold ($r_{tn}=0.9916$), leading to the acceptance of the alternative hypothesis that more than one group is present.

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

Drug description from package insert

Methylprednisolone sodium succinate for injection, USP is an anti-inflammatory glucocorticoid, which contains methylprednisolone sodium succinate as the active ingredient. Methylprednisolone sodium succinate, USP, is the sodium succinate ester of methylprednisolone, and it occurs as a white, or nearly white, odorless hygroscopic, amorphous solid. It is very soluble in water and in alcohol; it is insoluble in chloroform and is very slightly soluble in acetone (Dailymed, 2025).

The intravenous or intramuscular use of methylprednisolone sodium succinate for injection, USP is indicated for treatment of some allergic states, dermatologic diseases, endocrine disorders, gastrointestinal diseases, hematologic disorders, neoplastic diseases, nervous system diseases, ophthalmic diseases, renal diseases, respiratory diseases, and rheumatic disorders.



Figure 1. Photograph of the methylprednisolone sodium succinate drug product.

[Figure 1](#) is a photograph of 3 vials from lot 250102A. The lot numbers making up the spectral library were 220224A, 220504A, 220507A, 220508B, 220511A, 220514B, 220618B, 230525A, 230723B, 230729A, 231014A, 231019A, 231105A, 240223B, 240229A, 240303A, 240310A, 240311B, 240313A, 240602A, 240605A, 241031B, 241104A, 241221A, 241218B, and 250102A.

Background

Recent studies

Methylprednisolone, a potent synthetic glucocorticoid, has been a cornerstone of anti-inflammatory and immunosuppressive therapy for decades. Its utility spans a vast range of conditions, from chronic autoimmune diseases to acute inflammatory crises. However, recent years have seen a dynamic re-evaluation of its role, driven by large-scale clinical trials in new and established indications. Methylprednisolone has a complex therapeutic profile, where efficacy is highly dependent on patient selection, disease severity, timing, and dosage, challenging historical paradigms and refining its place in modern medicine. But methylprednisolone cannot do its intended work if it doesn't have the intended quality.

The COVID-19 pandemic served as an unprecedented catalyst for clinical research, forcing the medical community to rapidly evaluate existing therapies for a novel disease. The pathophysiology of severe COVID-19, characterized by a hyperinflammatory state or "cytokine storm" leading to acute respiratory distress syndrome (ARDS), provided a compelling rationale for investigating potent anti-inflammatory agents like corticosteroids ([Climente-Martí, 2021](#)). Despite this, initial guidance from the World Health Organization (WHO) was cautious, advising against the routine use of steroids. This position was informed by experiences with previous coronavirus outbreaks, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS), where corticosteroid use was associated with delayed viral clearance without clear clinical benefit ([Climente-Martí, 2021](#)).

The landscape shifted dramatically with the publication of the RECOVERY trial, which demonstrated a significant mortality benefit with low-dose dexamethasone in hospitalized patients requiring respiratory support ([Climente-Martí, 2021](#)). This landmark finding validated the corticosteroid approach and spurred intense investigation into the efficacy of other glucocorticoids, including methylprednisolone, which some researchers hypothesized might offer advantages due to its pharmacokinetic properties and potentially greater lung tissue penetration ([Joshi, 2021](#)).

A critical question that emerged was the optimal dosing of corticosteroids. A nationwide prospective cohort study conducted in Japan between 2020 and 2021 provided crucial, nuanced insights by analyzing data from 67,348 hospitalized patients with acute COVID-19. ([Moromizato, 2023](#)). To overcome a common pitfall in observational research, the study employed a marginal structural model, a sophisticated statistical method designed to control for time-dependent bias. This approach helps prevent the erroneous conclusion that a drug is ineffective simply because it is given to sicker patients later in their hospital course ([Moromizato, 2023](#)).

The study's findings revealed a striking, severity-dependent effect of methylprednisolone dosing. For the most critically ill patients—those already receiving invasive mechanical ventilation (iMV)—intravenous "pulse therapy" with high-dose methylprednisolone (defined as 500 mg or greater per day) was associated with a significantly lower risk of in-hospital mortality, with a hazard ratio (HR) of 0.59 (95% Confidence Interval [CI] 0.52-0.68) compared to patients not

receiving the drug. This mortality benefit was more pronounced than that observed with intermediate-to-high doses (40-250 mg intravenously), which had an HR of 0.80 (95% CI 0.71-0.89) ([Moromizato, 2023](#)).

In stark contrast, the same high-dose pulse therapy was associated with significant harm in patients who were not on mechanical ventilation. Among these iMV-free patients, pulse therapy led to a more than threefold increase in the risk of in-hospital mortality (HR 3.38; 95% CI 3.02-3.79). This powerful, bidirectional finding highlights the critical importance of patient stratification. The data suggest that while the potent anti-inflammatory effect of pulse-dose methylprednisolone may be necessary to counteract the profound hyperinflammation of established, severe ARDS requiring mechanical ventilation, this same level of immunosuppression may be detrimental in patients with less severe disease ([Moromizato, 2023](#)).

Building on the theme of patient selection, a 2021 study conducted in Spain sought to determine if the benefit of corticosteroids was more closely tied to the degree of systemic inflammation than to the need for respiratory support. Researchers hypothesized that identifying patients with severe systemic inflammation (SSI) via biomarkers could allow for a more tailored therapeutic approach. The study evaluated intermediate-to-high doses of methylprednisolone (0.5-1.0 mg/kg/day) in hospitalized COVID-19 patients with pneumonia and evidence of SSI, defined as having two or more elevated inflammatory biomarkers (e.g., C-reactive protein, ferritin, D-dimer) ([Climente-Martí, 2021](#)).

The results supported the hypothesis that the degree of hyperinflammation is a key determinant of treatment effect. A significant reduction in 28-day all-cause mortality was observed in subgroups of patients with a higher inflammatory burden, and this benefit was independent of their respiratory support needs. For instance, among patients with four or more altered inflammatory biomarkers, mortality in the methylprednisolone group was 9.3% compared to 30.4% in the control group ($P=.018$).¹ The benefit was even more pronounced in patients who both required respiratory support and had a high inflammatory burden. This study suggests that a biomarker-guided strategy could help clinicians identify the specific patient profiles most likely to benefit from methylprednisolone therapy, moving beyond a one-size-fits-all approach based solely on oxygen requirements ([Climente-Martí, 2021](#)).

The intense research activity during the pandemic led to the initiation of numerous other clinical trials to further clarify the role, optimal dose, and timing of methylprednisolone in COVID-19. These included the MetCOVID trial ([NCT04343729, 2025](#)), a study on heart rhythm (NCT04391439), and a trial combining methylprednisolone with heparin that was withdrawn due to unavailability of heparin (NCT04485429).

The results from these and other studies have been mixed, often complicated by variations in trial design, patient populations, and dosing regimens. The MetCOVID trial, a randomized, placebo-controlled study in Brazil, found that a 5-day course of low-dose methylprednisolone (0.5 mg/kg twice daily) did not reduce 28-day mortality in the overall population of patients with severe COVID-19.² However, a subsequent analysis of the trial data revealed an important

long-term benefit: patients who received methylprednisolone had significantly better pulmonary function, specifically a higher forced vital capacity (FVC), at a 120-day follow-up. This suggests that even a short course of corticosteroids may play a role in mitigating the long-term fibrotic lung damage that can follow severe COVID-19 pneumonia ([Barros, 2021](#)).

Other meta-analyses and quasi-experimental studies have yielded a complex picture. Some have suggested that methylprednisolone may be superior to dexamethasone, while others have found them to be equivalent ([Porta, 2022](#)). A 2022 meta-analysis concluded that, compared with no glucocorticoids, methylprednisolone was associated with reduced short-term mortality and a lower need for mechanical ventilation. It further suggested that patients with severe COVID-19 were most likely to benefit from a short-term (≤ 7 days), low-dose (≤ 2 mg/kg/day) regimen ([Joshi, 2022](#)). These varying conclusions underscore the difficulty in synthesizing evidence from heterogeneous studies and reinforce the central theme that the drug's effect is highly context-dependent.

Recalls

Recent years have seen several significant recalls of injectable methylprednisolone products, shedding light on vulnerabilities in the pharmaceutical manufacturing and supply chain. These events, ranging from particulate contamination to potential sterility breaches, underscore the critical importance of stringent quality control in the production of sterile injectable drugs. None of these recalls involve Tianjin Kingyork Pharmaceuticals, but recalls of other manufacturers' products can put pressure on the total supply and lead to shortages of drugs not subject to recalls.

Sagent has faced quality issues with its methylprednisolone products ([FDA, 2018](#)). In March 2018, the company conducted a voluntary nationwide recall of ten lots of methylprednisolone sodium succinate for injection across multiple strengths (40 mg, 125 mg, and 1 g vials). This earlier recall was triggered by the discovery of high out-of-specification impurity results during routine quality testing of stability samples. While the specific impurity was not identified, the potential clinical consequence was a decrease in the product's effectiveness. These products were manufactured for Sagent by Gland Pharma Ltd., another firm based in India, highlighting the global nature of the pharmaceutical supply chain.

Shortages

Drug shortages are a chronic and worsening problem in the U.S. healthcare system, and injectable methylprednisolone is frequently affected. These shortages disrupt patient care, increase costs for hospitals, and force clinicians to make difficult therapeutic decisions. The instability of the methylprednisolone supply is not a new issue but rather a long-standing challenge rooted in a combination of demand shocks, manufacturing fragility, and market economics.

The American Society of Health-System Pharmacists (ASHP), a primary source for drug shortage information, consistently reports supply disruptions for injectable methylprednisolone

from multiple major manufacturers. According to recent ASHP bulletins, several key presentations of methylprednisolone sodium succinate for injection are on backorder, with resolution dates stretching well into 2025 ([ASHP, 2025](#)). None of these currently involve Tianjin Kingyork Pharmaceuticals.

Pfizer, the manufacturer of the brand-name product Solu-Medrol, has its 500 mg vials on backorder with an estimated release date of June 2025. The commonly used 40 mg and 125 mg Act-O-Vial presentations are available only in limited supply, with allocations released on a weekly basis. Fresenius Kabi has its 40 mg vials of methylprednisolone sodium succinate on backorder, with an estimated release date of late-June to early-July 2025. Hikma Pharmaceuticals is experiencing backorders across its product line, with 40 mg, 125 mg, and 1 g vials all affected. The company has provided staggered estimated release dates ranging from mid-June to mid-September 2025 for these different strengths.

FDA Medwatch

An FDA Form 3500 Medwatch describing the findings of this Rapid Communication was filed.

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected from inventory as part of routine medication quality screening. A representative sample of individual vials were selected for screening and noted to be stored under the conditions required by the manufacturer in their original packaging. FTNIR spectra were collected noninvasively and nondestructively through the bottom of the vials using a Thermo Scientific Antaris II FTNIR Analyzer (Waltham, MA, USA)([Isaacs, 2023b](#)).

Smoothing

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

Multiplicative Scatter Correction (MSC)

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

BEST (Bootstrap Error-Adjusted Single-sample Technique)

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

Principal Components (PCs)

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

Subcluster Detection

In typical near-infrared multivariate statistical analyses, samples with similar spectra produce points that cluster in a certain region of spectral hyperspace. These clusters can vary significantly in shape and size due to variation in sample packings, particle-size distributions, component concentrations, and drift with time. These factors, when combined with discriminant analysis using simple distance metrics, produce a test in which a result that places a particular point inside a particular cluster does not necessarily mean that the point is actually a member of the cluster. Instead, the point may be a member of a new, slightly different cluster that overlaps the first. A new cluster can be created by factors like low-level contamination, moisture uptake, or instrumental drift. An extension added to part of the BEST, called FSOB (Fast Son of BEST) can be used to set nonparametric probability-density contours inside spectral clusters as well as

outside ([Isaacs, 2023c](#))([Lodder, 1988](#)), and when multiple points begin to appear in a certain region of cluster-hyperspace the perturbation of these density contours can be detected at an assigned significance level using r values, and visualized using quantile-quantile (QQ) plots. The detection of unusual samples both within and beyond 3 SDs of the center of the training set is possible with this method. Within the ordinary 3 SD limit, however, multiple instances are needed to detect unusual samples with statistical significance.

Artificial Intelligence Tools

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

Results and Discussion

Intralot analysis

[Figure 2](#) shows the smoothed spectral graphs for the samples from one lot (250102A) of methylprednisolone sodium succinate screened for intralot variation.

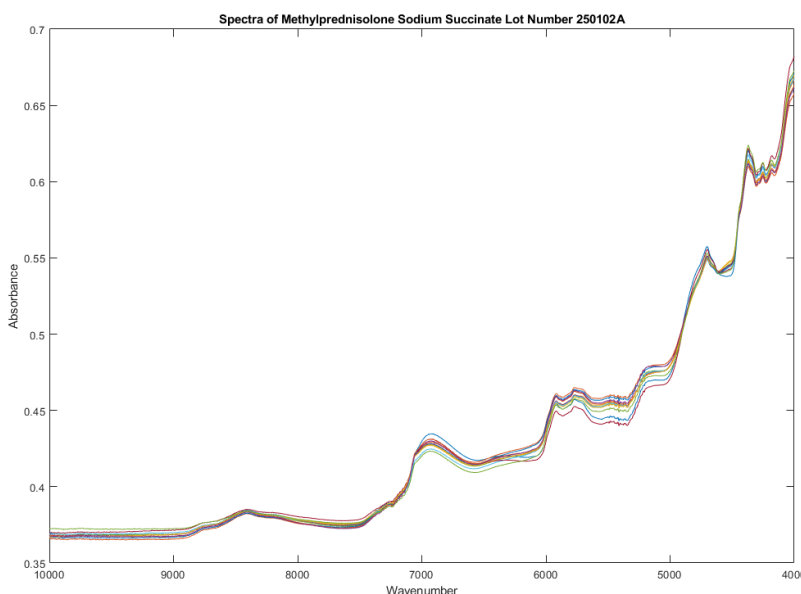


Figure 2. Full spectra of samples of methylprednisolone sodium succinate from lot number 250102A. Differences between the spectra are obvious in the ranges from 4500 to 5000 cm^{-1} and 6000 to 6500 cm^{-1} .

[Figure 3](#) depicts the spectra of the same samples shown in [Figure 2](#), zoomed in on the region from 7000 to 8500 cm^{-1} .

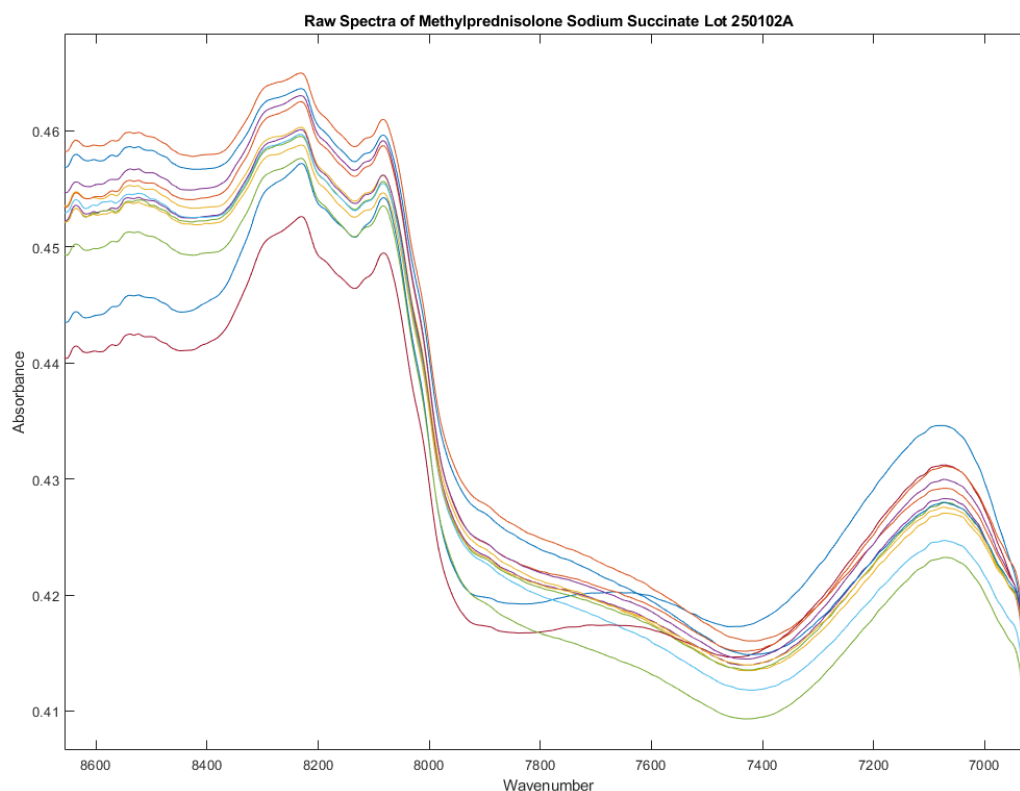


Figure 3. Spectra of the same samples (lot 25010028) in [Figure 2](#), zoomed in on the region from 7000 to 8500 cm^{-1} . The two spectra that appear to be most unusual (shown as the blue and violet curves) are 4.4 and 5.9 SDs from the center of the cluster of samples.

The analysis of a single lot of methylprednisolone sodium succinate revealed significant intra-lot variability in the near-infrared spectra. Differences were visually apparent in the absorbance peaks, particularly in the ranges of 4500 to 5000 cm^{-1} and 6000 to 6500 cm^{-1} (see [Figure 2](#)). Further examination of a zoomed-in region (7000 to 8500 cm^{-1} , see [Figure 3](#)) showed two spectra that were statistically significant outliers, positioned at 4.4 and 5.9 standard deviations (SDs) from the center of the sample cluster. A principal component (PC) score plot of the spectra (see [Figure 4](#)) also identified vials 1 and 7 as being outside the main elliptical cluster, while vial 12, despite a larger Euclidean displacement, had a smaller distance in terms of standard deviations because its displacement occurred along the major axis of the ellipsoid. These findings suggest a lack of content uniformity within this single manufacturing lot. As a result, 25 additional lots of methylprednisolone sodium succinate from the spectral library were examined to determine interlot variability.

PC Plots for Intralot Analysis

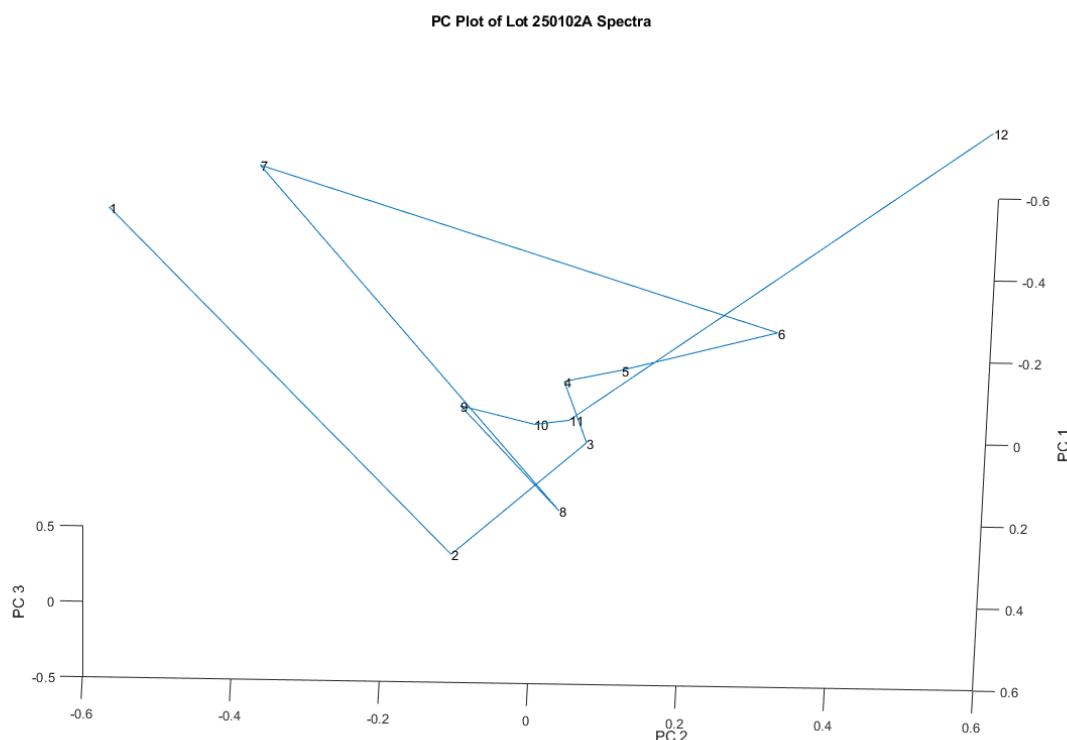


Figure 4. The principal component score plot for the spectra in [Figure 2](#). All of the vials except 1 and 7 appear to belong to the same elliptical cluster. Vial 12 is displaced farther from the center of the ellipsoid than vials 1 or 7 in terms of the Euclidean metric. However, the displacement occurs along the major axis of the ellipsoid so the distance in standard deviations is actually smaller for vial 12 than for vial 1 and vial 7.

[Table 1](#) provides data on the cumulative variation explained by each principal component (PC) for the spectra of a single lot (250102A) of methylprednisolone sodium succinate.

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

PC Number	Variation in this PC	Cumulative PC Variation
1	0.6911	0.6911
2	0.2012	0.8923
3	0.0725	0.9648
4	0.0251	0.9900
5	0.0058	0.9958
6	0.0028	0.9986
7	0.0008	0.9994
8	0.0003	0.9997
9	0.0001	0.9998

By the fourth principal component, 99% of the total variance is explained. Typically the first principal component in a well-controlled drug manufacturing process is responsible for 90% or more of the total spectral variation of the drug product. In this case, the first principal component only captures 69% of the total spectral variation.

Interlot analysis

Smoothed spectral graphs of methylprednisolone library

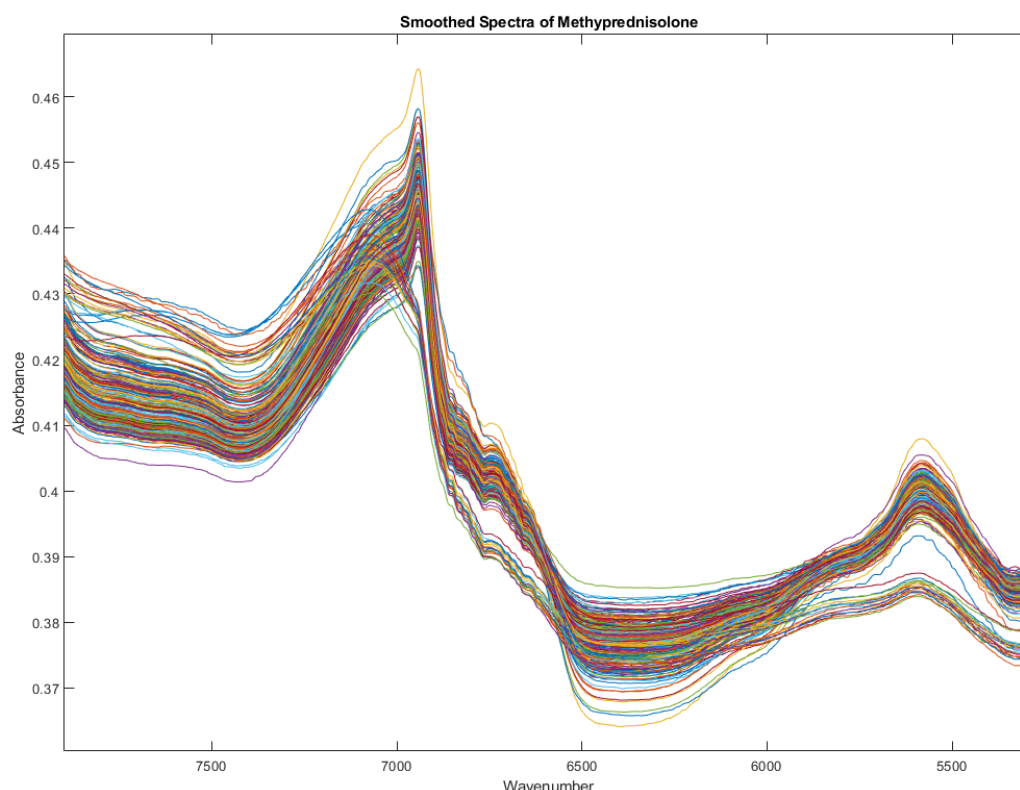


Figure 5. Zoom-in on the spectra of 242 vials of methylprednisolone sodium succinate, obtained from the spectral library composed of samples from 26 lots of the drug. Obvious differences in peaks are seen around 5600 and 7000 cm^{-1} , where two different groups of spectra can be observed. For example, one group of spectra has a sharp peak at about 6900 cm^{-1} , while the other group has a rounded peak at about 7100 cm^{-1} .

[Figure 5](#), obtained from the spectral library composed of samples from 26 lots of the drug, shows obvious differences in the peaks of the spectra, particularly around 5600 cm^{-1}

and 7000 cm^{-1} . The image reveals two distinct groups of spectra. For example, one group has a sharp peak at approximately 6900 cm^{-1} , while the other has a more rounded peak at about 7100 cm^{-1} . This visual evidence indicates significant inter-lot variability, suggesting that the manufacturing process for these different lots may not have been consistent.

PC Plots

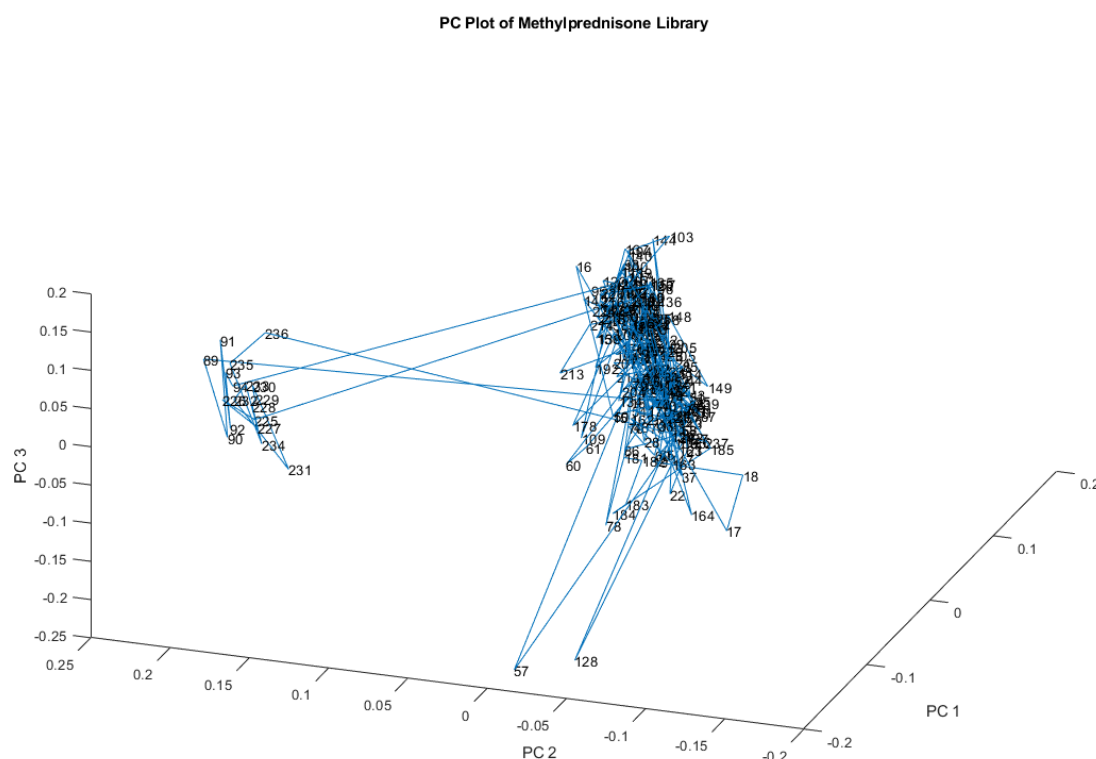


Figure 6. Scatterplot of the first three principal components of the spectral library of 242 vials of methylprednisolone. Two large groups are observed, with a couple of potential outliers from the larger group (vials 57 and 128) appearing on the right.

[Figure 6](#) shows a clear division of the spectral data into two large groups or clusters. The plot also identifies potential outliers from the larger group, specifically vials 57 and 128, which appear on the right side of the main cluster. This visualization supports the finding that there is significant variability across different manufacturing lots of the drug, as the spectra do not form a single, cohesive cluster typical of a well controlled pharmaceutical manufacturing process. The presence of these two distinct clusters confirms the conclusion from the subcluster detection algorithm that two groups exist in the spectral data. The vials are scanned and connected in order by blue lines. Four blue lines connect the two clusters in [Figure 6](#), suggesting that the

samples from the smaller cluster come from two or three different lots, and are not scattered through all of the lots.



Figure 7. Scatterplot of principal components 4, 5, and 6 of the spectral library of 242 vials of methylprednisolone. These are the same spectra depicted in [Figure 6](#). The potential outliers in [Figure 7](#) are vials 90, 92, 225 and 231.

[Figure 7](#) shows a scatterplot of the same spectra as in [Figure 6](#), but from PCs representing smaller spectral variations. This view reveals a new set of potential outliers: vials 90, 92, 225, and 231. The plot further confirms the complexity and significant variability of the drug's spectral data across different lots by identifying different outliers when viewed along different principal components.



Figure 8. Scatterplot of principal components 7, 8 and 9 of the spectral library of 242 vials of methylprednisolone. These are the same spectra depicted in [Figure 6](#). The main group appears almost toroidal in this view (compare to [Figure 9](#)).

[Figure 8](#) provides an even more detailed view of the inter-lot variability. While the main group of vials appears to be a single cluster in the PC 1-3 plot ([Figure 6](#)), this view along higher-order principal components (PC 7, 8, and 9) reveals that this seemingly single cluster is actually shaped like a toroid, or a doughnut, with about five outliers appearing on PC7 to the right of the group. The scatterplot suggests there may be more individual vials with unique spectral characteristics. This toroidal shape further supports the conclusion of significant and complex variability in minor constituents among the drug lots.

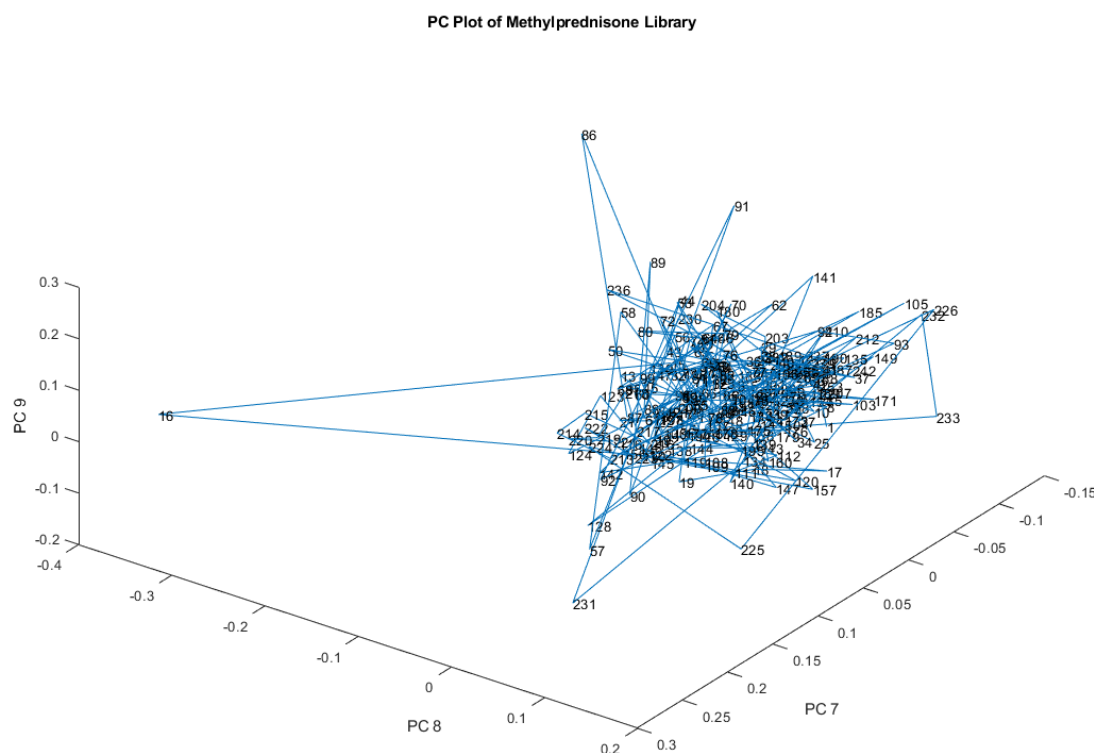


Figure 9. Another scatterplot of principal components 7, 8 and 9 of the spectral library of 242 vials of methylprednisolone, rotated from the view in [Figure 8](#). Vial 16, and perhaps vial 66, appear to be the outliers.

[Figure 9](#) is another scatterplot of principal components (PCs) 7, 8, and 9 for the spectral library of methylprednisolone vials. This plot is a rotated view of the same data shown in [Figure 8](#). This rotation is an edge-on view of the torus, so the hole in the center is not visible.

This different perspective highlights additional potential outliers that were not as obvious in the previous view, specifically vials 16 and 66. By showing the data from a new angle, [Figure 9](#) reinforces the finding that there is complex, multidimensional variability among the different lots of methylprednisolone, as the data points do not form a single, tight cluster.

Subcluster Detection and QQ plotting

The sample vials in smaller group on the left side of [Figure 6](#) are vials 89, 90, 91, 92, 93, 94, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, and 236. The subcluster detection algorithm was run on the 242 vials in the spectral library of methylprednisolone. The 98% confidence level on the main group was $r_{tn}=0.9916$. The measured $r_{ts}=0.8704$, so the alternative hypothesis was accepted and two groups exist in the spectral data. A QQ plot from the

subcluster Detection test appears in [Figure 10](#). There are 55 SDs between the two groups using the subcluster detection correlation metric.

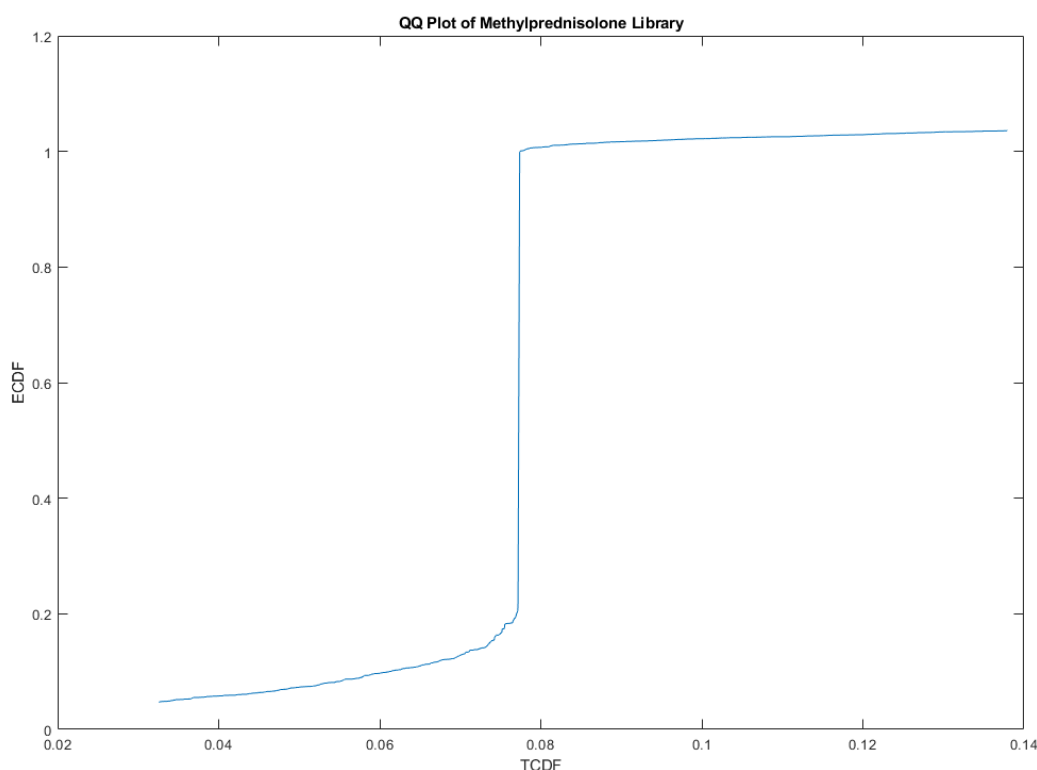


Figure 10. QQ plot from the subcluster detection algorithm ($r_{tn}=0.99$, $r_{ts}=0.87$, $p=0.02$) showing two groups are present in the spectral library.

[Table 2](#) provides a breakdown of how much of the total variance across the entire spectral library (composed of 242 vials from 26 lots) is explained by each principal component (PC). In [Table 2](#), PC1 accounts for the largest portion of the variance at 64.86%. The first three PCs combined explain over 95% of the total variation in the spectral library. By PC6, 99% of the variance is explained. [Table 1](#) describes the PC variation for a single lot of methylprednisolone (lot number 250102A), while [Table 2](#) describes the PC variation for the entire spectral library (26 lots). The primary difference between the tables lies in how quickly the cumulative variance reaches 99% or more. The data for the single lot ([Table 1](#)) shows that the first four PCs explain 99% of the variance. In contrast, the data for the full library ([Table 2](#)) requires the first six PCs to explain 99.00% of the variance. The amount of variation captured by table one is still remarkably similar to that of table 2, suggesting that the one lot actually reflects the amount of variation in the 26 lots rather well.

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	0.6486	0.6486
2	0.2643	0.9129
3	0.0419	0.9548
4	0.0187	0.9735
5	0.0085	0.9820
6	0.0080	0.9900
7	0.0047	0.9947
8	0.0027	0.9974
9	0.0009	0.9983

PC Loadings Plots

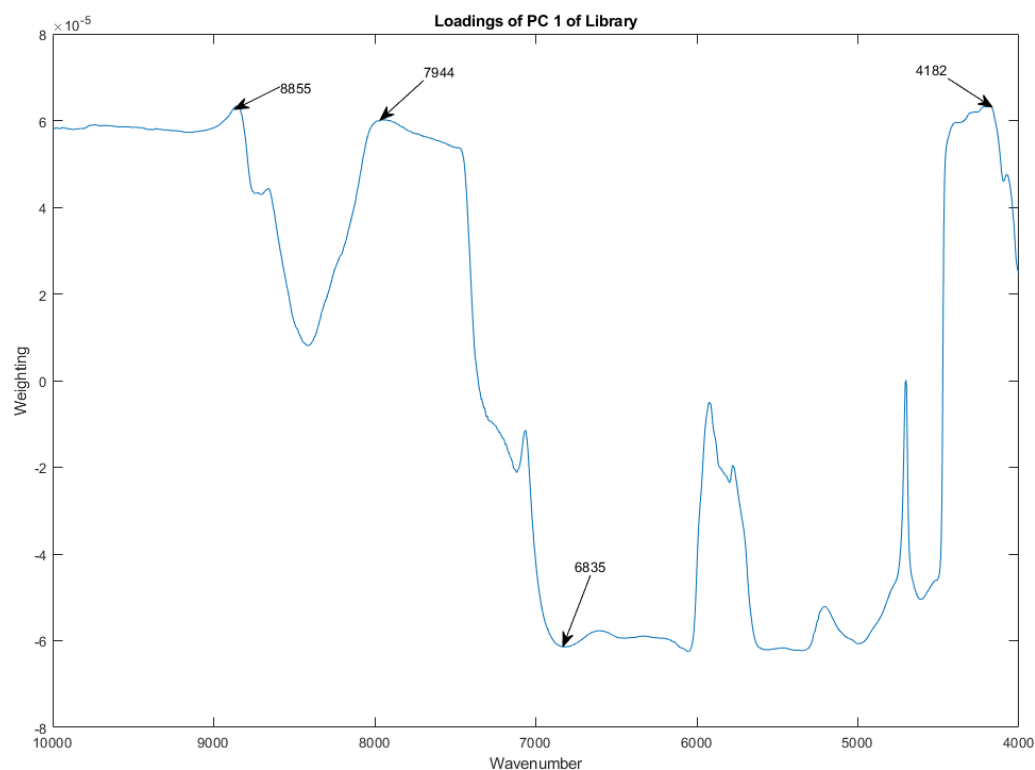


Figure 11. Principal component loadings spectrum of the vials in the spectral library for PC1. Important spectral features are noted at 4182, 6835, 7944, and 8855 cm^{-1} .

[Figure 11](#) is a principal component (PC) loadings spectrum for the first principal component (PC1) of a spectral library of 242 vials of methylprednisolone sodium succinate. Loadings plots show the relationship between variables (in this case, wavenumbers) and the principal components.

Specifically, [Figure 11](#) shows that the most significant spectral features for PC1 are at 4182, 6835, 7944, and 8855 cm^{-1} . These features are the most important for explaining the variability in the data that is captured by PC1. The values on a loadings plot (the y-axis) indicate the weighting of each wavenumber in the linear combination that forms the principal component.

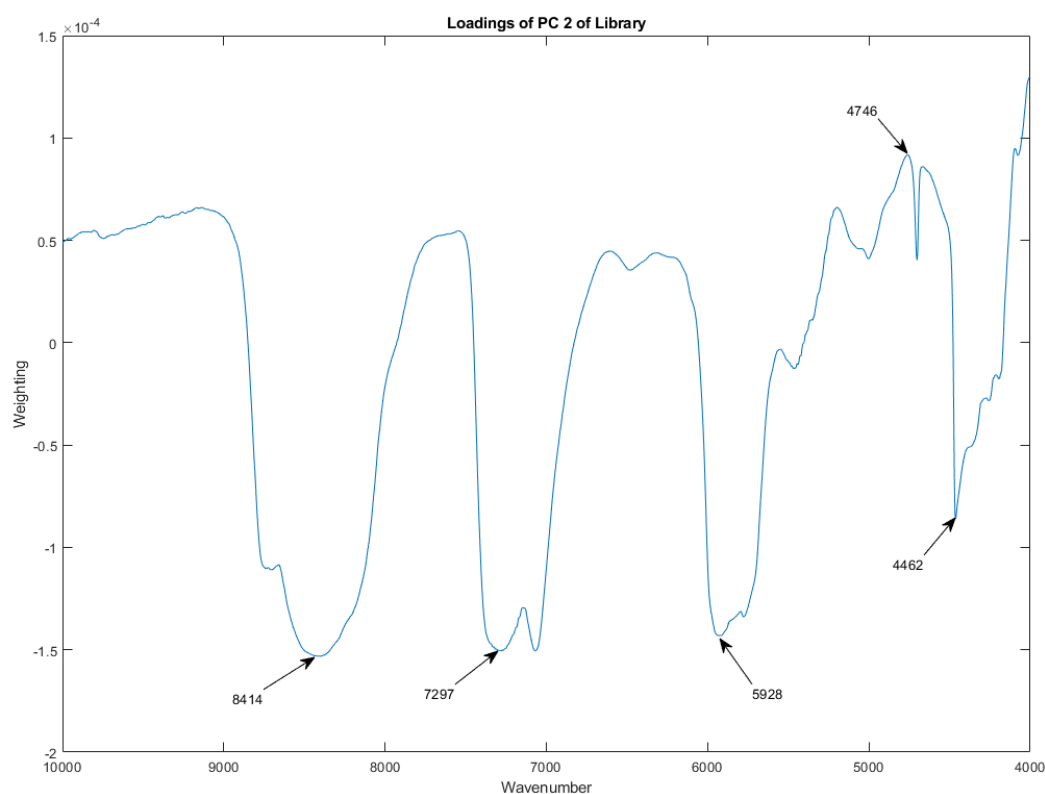


Figure 12. Principal component loadings spectrum of the vials in the spectral library for PC2. Important spectral features are noted at 4462, 5928, 7297, and 8414 cm^{-1} .

[Figure 12](#) is a principal component (PC) loadings spectrum for the second principal component (PC2) of the spectral library of methylprednisolone vials. It highlights the wavenumbers that contribute most to the variance captured by PC2. The most significant spectral features for PC2 are at 4462, 5928, 7297, and 8414 cm^{-1} . These are the features that are most heavily weighted in the calculation of PC2.

The spectral features noted in [Figure 12](#) correspond to specific molecular vibrations within the methylprednisolone sodium succinate molecule or any excipients added to the drug product. These wavenumbers can be assigned to overtones and combination bands of fundamental vibrations, primarily from C-H bonds. The 8414 cm^{-1} peak is in the second overtone region of C-H stretching vibrations. It likely corresponds to the second overtone of stretching from methyl (CH_3) or methylene (CH_2) groups present in the steroid structure of methylprednisolone. The 7297 cm^{-1} feature falls within the first overtone region of C-H stretching vibrations. More specifically, it is characteristic of the first overtone of methyl (CH_3) C-H stretching. The 5928 cm^{-1} band is also in the first overtone region for C-H stretching vibrations. It is typically associated with the C-H stretching in methyl and methylene groups. The 4462 cm^{-1} peak is located in the combination band region. Absorptions in this area arise from a combination of fundamental vibrations. This specific feature is likely due to the combination of C-H stretching and C-H deformation (bending) vibrations from methylene groups in the molecule. Because [Figure 12](#) is a loadings spectrum, the variations observed in the principal component loadings at these specific wavenumbers highlight inconsistencies in the vibrational states of the C-H bonds throughout the samples, which supports the conclusion of intralot and interlot variability.

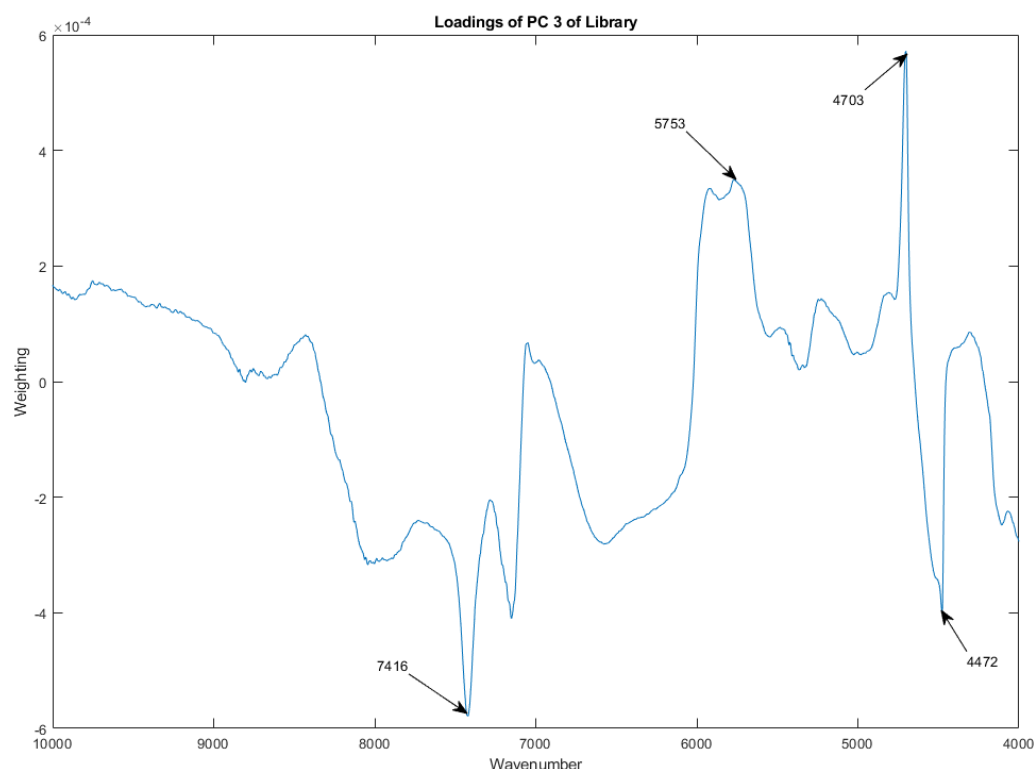


Figure 13. Principal component loadings spectrum of the vials in the spectral library for PC3. Important spectral features are noted at 4472, 4703, 5753, and 7416 cm^{-1} .

[Figure 13](#) is a principal component (PC) loadings plot that identifies the specific spectral features responsible for the third largest source of variation (PC3) among the scanned methylprednisolone samples. A loadings plot shows the weight or importance of each variable (in this case, each wavenumber) for a given principal component. The key takeaways from [Figure 13](#) are:

- **Identifies Key Wavenumbers:** The plot highlights that variations at wavenumbers 4472, 4703, 5753, and 7416 cm^{-1} are particularly influential in describing the differences between samples along the PC3 axis. The large positive and negative "weighting" values at these points indicate their significance.
- **Explains Sample Separation:** This plot helps explain *why* samples are separated in the 3D PC scores plots (like [Figure 6](#)). The differences observed at these specific wavenumbers are what cause the samples to spread out along the PC3 axis.
- **Characterizes Subtle Variation:** While PC1 and PC2 account for over 91% of the total variance, PC3 still explains 4.19% of the remaining variability in the spectral library. Therefore, [Figure 13](#) points to the chemical or physical properties that cause more subtle, but still significant, differences among the drug samples.

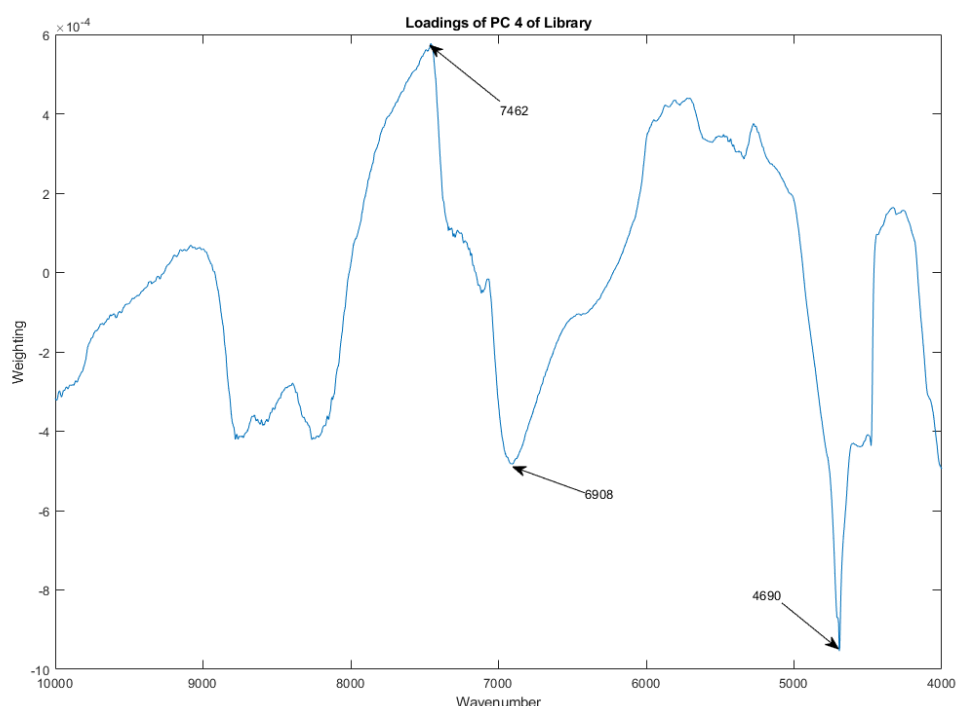


Figure 14. Principal component loadings spectrum of the vials in the spectral library for PC4. Important spectral features are noted at 4690, 6908, and 7462 cm^{-1} .

[Figure 14](#) is a principal component (PC) loadings plot that pinpoints the specific spectral features causing the fourth largest source of variation among the scanned vials. This plot shows the "weighting" or importance of each wavenumber for the fourth principal component (PC4). The key information it provides is:

- **Identifies Influential Wavenumbers:** The plot and its caption specifically note that variations in the spectra at 4690, 6908, and 7462 cm^{-1} are the primary drivers of the variance captured by PC4. The large peaks and valleys at these points indicate their high importance.
- **Explains Subtle Differences:** PC4 accounts for 1.87% of the total spectral variation in the library. While this is less than the first few PCs, it represents a more subtle level of chemical or physical difference between the samples. [Figure 14](#) tells us exactly which parts of the spectral fingerprint are changing to cause this variation.
- **Correlates to Sample Outliers:** This loadings plot provides the reason for the sample separation seen in other figures. For instance, [Figure 7](#), which plots the PC scores for PCs 4, 5, and 6, reveals a new set of potential outliers. [Figure 14](#) explains that it is the unique spectral behavior at the identified wavenumbers (4690, 6908, and 7462 cm^{-1}) that causes those specific vials to be separated from the main cluster along the PC4 axis.

[Figure 15](#) is a principal component (PC) loadings plot that identifies the specific spectral features responsible for the fifth largest source of variation among the vials. [Figure 15](#) and its caption highlight that the spectral variations at 4465, 5205, and 6802 cm^{-1} are the main contributors to the variance captured by PC5. The peaks and valleys in the plot at these points show their high "weighting" or influence. The fifth principal component (PC5) accounts for a small fraction of the total spectral variation, just 0.85%. This plot pinpoints the exact wavenumbers responsible for these very subtle chemical or physical differences among the samples. This plot helps explain the arrangement of samples in the PC scores plots. For example, in [Figure 7](#), which plots scores for PCs 4, 5, and 6, any separation or clustering of vials along the PC5 axis is a direct result of differences in their spectra at the specific wavenumbers identified in [Figure 15](#). This helps to characterize complex patterns and identify outliers that aren't apparent in the first few, more dominant, principal components.

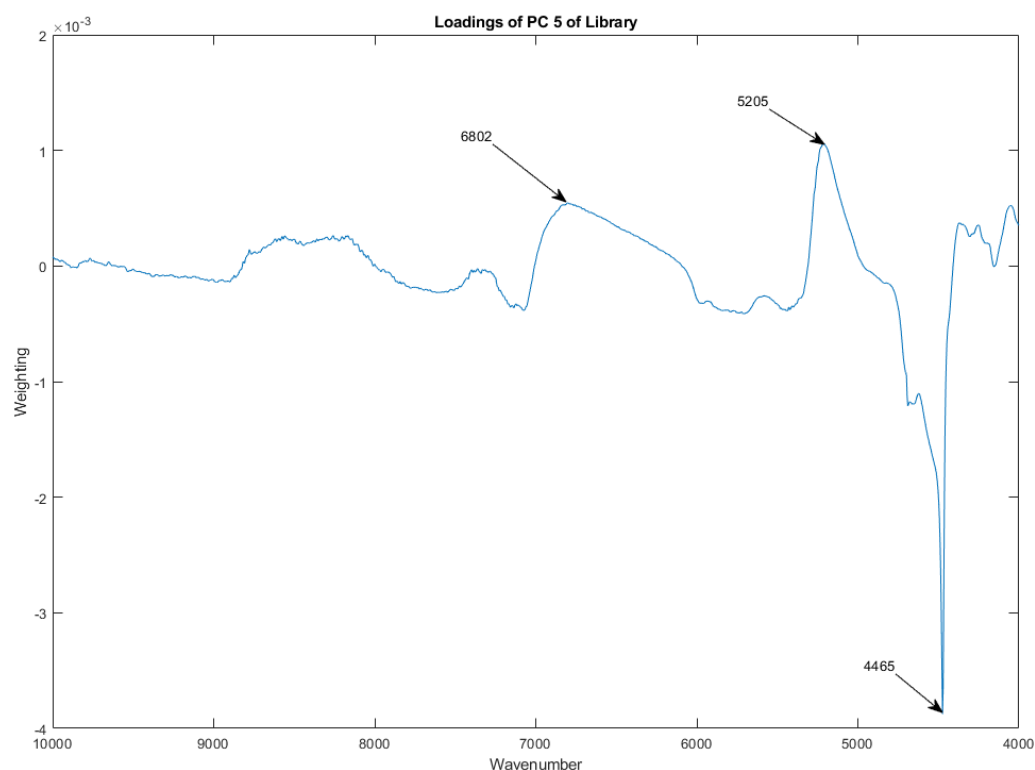


Figure 15. Principal component loadings spectrum of the vials in the spectral library for PC5. Important spectral features are marked at 4465, 5205, and 6802 cm^{-1} .

[Figure 16](#) identifies important spectral features at 4479, 4696, 7125, and 7436 cm^{-1} . The 7436 cm^{-1} absorption is in the first overtone region. It is characteristic of the first overtone of C-H stretching vibrations, likely from methyl (CH_3) or methylene (CH_2) groups in the molecule's steroid backbone. The 7125 cm^{-1} band is in the first overtone region. This feature is another indicator of the first overtone of C-H stretching vibrations. The presence of multiple peaks in this region suggests C-H bonds in slightly different chemical environments within the molecule. The 4696 cm^{-1} peak falls within the combination band region. It arises from a combination of C-H stretching and C-H deformation (bending) vibrations. The 4479 cm^{-1} feature is also a combination band, this feature is typically assigned to the combination of C-H stretching and bending vibrations, most likely from a methylene (CH_2) group.

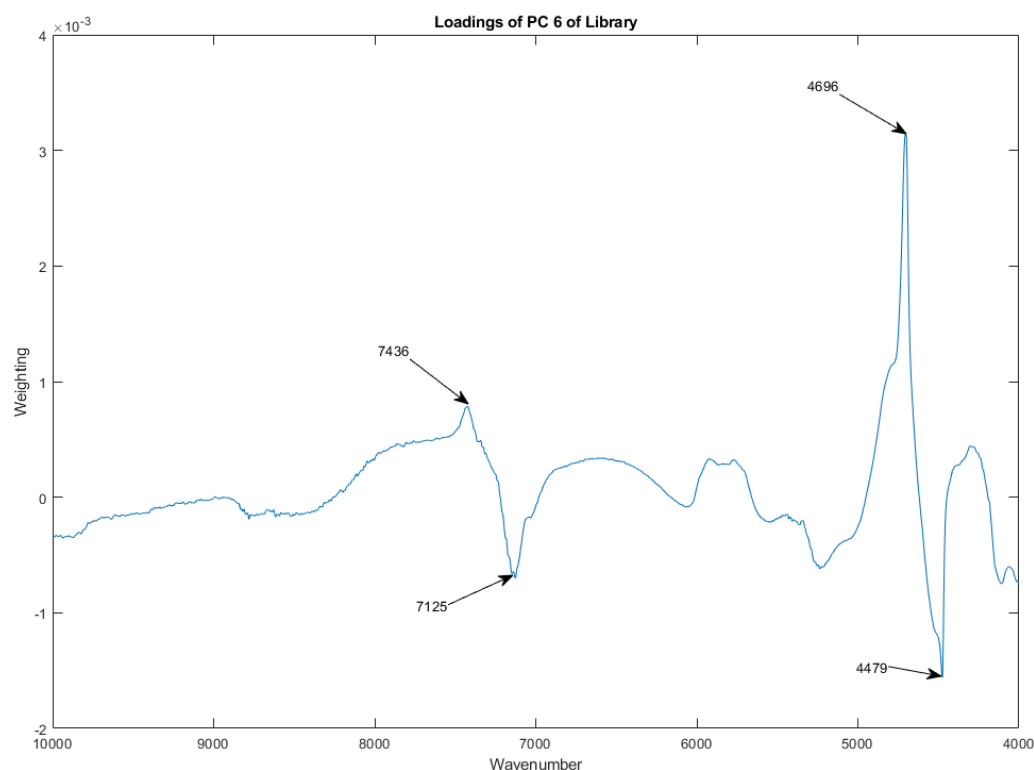


Figure 16. Principal component loadings spectrum of the vials in the spectral library for PC6. Important spectral features are noted at 4479, 4696, 7125, and 7436 cm^{-1} .

[Figure 17](#) is a loadings plot that identifies the specific spectral features responsible for the seventh largest source of variation (PC7) among the scanned vials. The plot and its caption highlight that the most important spectral features for PC7 are located at wavenumbers 4281, 5205, 5396, 6076, and 7125 cm^{-1} . The high and low "weighting" values at these points show their significance. PC7 accounts for a very small amount of the total variation (0.47%). However, examining these higher-order components is crucial for understanding complex patterns. This loadings plot provides the reason for the sample arrangements seen in the scores plots of higher-order PCs. For instance, the analysis of PCs 7, 8, and 9 revealed that the main cluster of samples has a toroidal (doughnut) shape and that about five outliers appear along the PC7 axis. [Figure 17](#) explains that the unique spectral behavior at the identified wavenumbers is what separates those specific outliers from the main group.

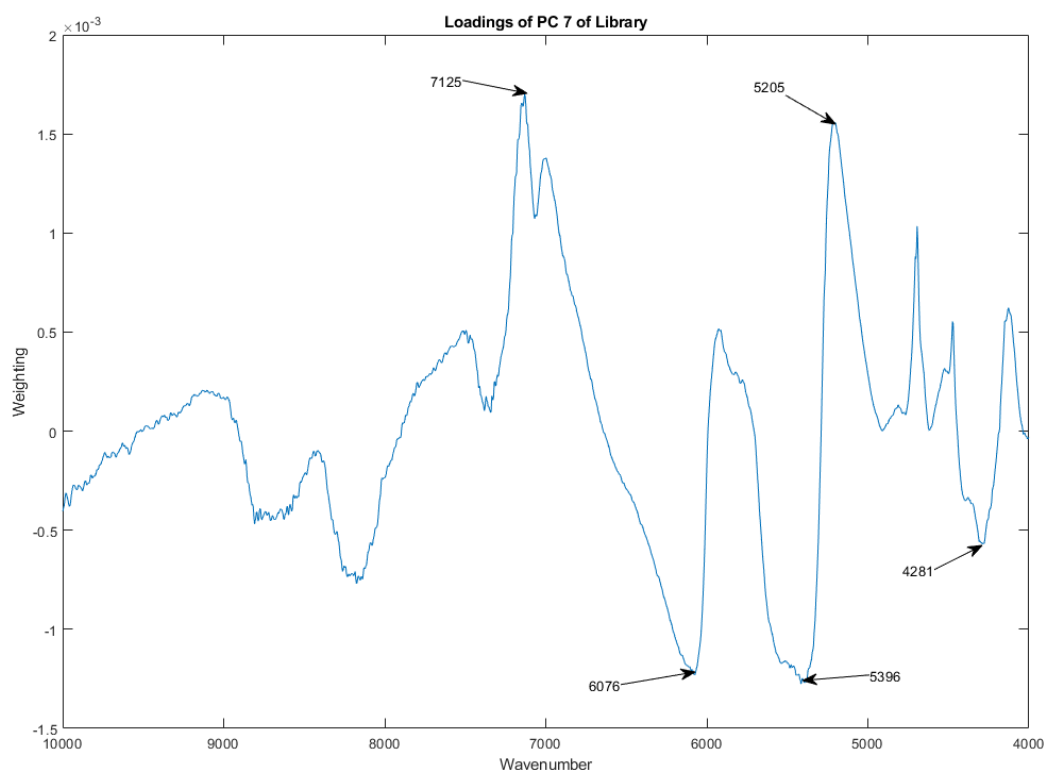


Figure 17. Principal component loadings spectrum of the vials in the spectral library for PC7. Important spectral features are noted at 4281, 5205, 5396, 6076, and 7125 cm^{-1} .

[Figure 18](#) is a loadings plot for PC8. Its primary importance lies in how it contributes to the study's overall conclusion of complex variability. The plot shows that variations at 4465, 4690, 5191, 6980, and 8723 cm^{-1} are the main drivers for the variance captured by PC8. PC8 accounts for only 0.27% of the total variance in the samples. A small amount of spectral noise begins to appear in the spectra, starting at the high wavenumber end of the spectrum. While this PC represents only a tiny fraction of the overall variation, analyzing it is crucial for a complete understanding of the data. This plot helps lead to the chemical basis for the complex, multi-dimensional shapes, such as the toroidal structure of the main sample cluster, which is only visible when examining these higher-order components (as seen in [Figure 8](#)). The plot's main function is to translate the abstract mathematical separation seen in the PC scores plots back to tangible chemical information. Any separation of samples along the PC8 axis is a direct result of physical or chemical differences that affect how the molecules vibrate at the wavenumbers identified in this figure.

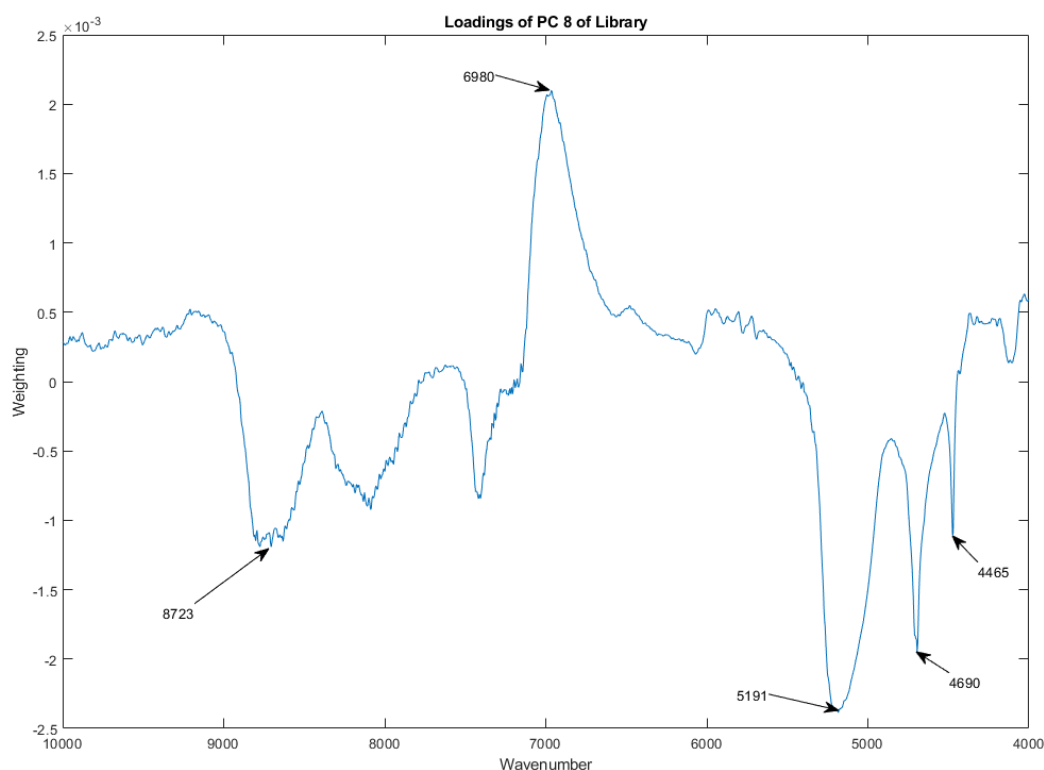


Figure 18. Principal component loadings spectrum of the vials in the spectral library for PC8. Important spectral features are marked at 4465, 4690, 5191, 6980, and 8723 cm^{-1} .

[Figure 19](#) is a loadings plot that shows which specific spectral features are responsible for the ninth largest source of variation (PC9) among the scanned vials. A loadings plot helps connect the abstract mathematical components from a Principal Component Analysis (PCA) back to real, physical data. The plot highlights that variations at 4393, 4690, 5588, and 6960 cm^{-1} are the most influential drivers for the variance captured by PC9. PC9 represents a tiny fraction of the total variation, accounting for only 0.09% of the variance in the entire spectral library. This plot pinpoints the exact source of this highly subtle difference among the samples. The plot provides a chemical explanation for the arrangement of samples in the 3D scores plots that use higher-order components (like [Figure 8](#) and [Figure 9](#)). Any separation of vials along the PC9 axis in those plots is a direct result of differences in their spectra at the specific wavenumbers identified here.

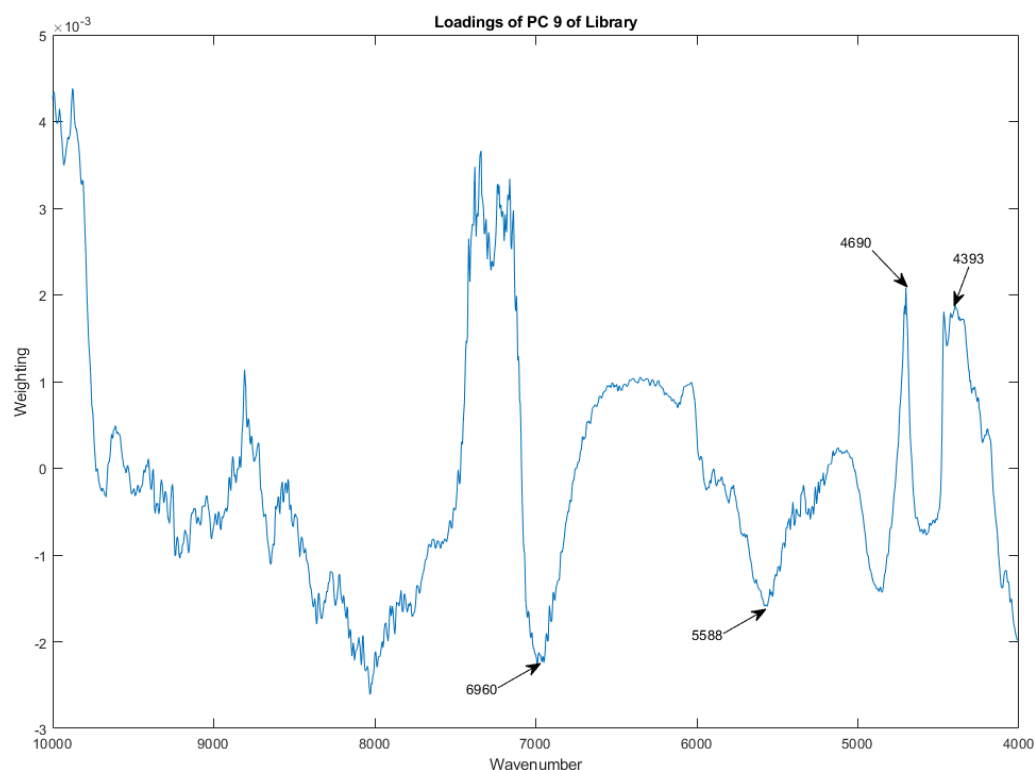


Figure 19. Principal component loadings spectrum of the vials in the spectral library for PC9. Important spectral features are noted at 4393, 4690, 5588, and 6960 cm^{-1} .

Conclusion

Methylprednisolone sodium succinate for injection, USP is an anti-inflammatory glucocorticoid. The active ingredient is methylprednisolone sodium succinate, which is the sodium succinate ester of methylprednisolone. The substance is a white or nearly white, odorless, hygroscopic, amorphous solid. It is highly soluble in water and alcohol, insoluble in chloroform, and only slightly soluble in acetone. This injectable form of the drug is indicated for the treatment of various conditions, including some allergic states, dermatologic, endocrine, gastrointestinal, hematologic, neoplastic, nervous system, ophthalmic, renal, respiratory, and rheumatic disorders.

The analysis of a single lot of methylprednisolone sodium succinate revealed significant intra-lot variability in the near-infrared spectra. Differences were visually apparent in the absorbance peaks, particularly in the ranges of 4500 to 5000 cm^{-1} and 6000 to 6500 cm^{-1} . Further examination of a zoomed-in region (7000 to 8500 cm^{-1}) showed two spectra that were

statistically significant outliers, positioned at 4.4 and 5.9 standard deviations (SDs) from the center of the sample cluster.

Examination of the entire spectral library revealed significant differences between manufacturing lots. The smoothed spectra of all 242 vials showed obvious differences, particularly in the peak structures around 5600 cm^{-1} and 7000 cm^{-1} . The spectra appeared to form two distinct groups; for example, one group displayed a sharp peak around 6900 cm^{-1} , while the other had a more rounded peak near 7100 cm^{-1} . This visual evidence suggests that the manufacturing process was not consistent across the different lots.

PC plots, which visualize the variance in the data, confirmed the presence of multiple groups and outliers. A plot of the first three principal components (PCs) showed the data was clearly divided into two large clusters rather than a single cohesive one, which would be expected from a well-controlled process. Two vials were identified as potential outliers in this view. When viewing plots of higher-order PCs (PCs 4, 5, and 6), which represent more subtle variations, a new set of 4 potential outliers emerged. Examination of PCs 7, 8, and 9 revealed even more complexity. The main cluster, which appeared as a single group in the first PC plot, was shown to have a toroidal, or doughnut-like, shape. This view highlighted additional outliers, including vials 16 and 66.

To statistically validate the visual findings, a subcluster detection algorithm was applied to the spectral data from the 242 vials. The algorithm confirmed that two distinct groups exist within the spectral data. The measured correlation value ($r_{ts}=0.8704$) was below the 98% confidence level threshold ($r_{tn}=0.9916$), leading to the acceptance of the alternative hypothesis that more than one group is present. A QQ plot provided a visual representation of the algorithm's output, clearly showing the presence of two groups.

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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