

Fourier Transform Near-Infrared Spectrometry Reveals Significant Inter-Lot Variability in Thyrotropin Alfa (Thyrogen®) Quality

Uiyeol Yoon^{1,2}, Ayumi Tariant³, Philip J. Almeter^{1,2}, Eleonora Hasani^{1,2}, Aaron N. Hunter¹, Thomas A. Lyman¹, Stephanie P. Zapata¹, Bradley S. Henderson¹, Seth A. Larkin^{1,2}, Adler Crumrin^{1,2}, Jerod Smith^{1,2}, Spencer Pergrem^{1,2}, Ashton Plymale^{1,2}, Bailee Ramnes^{1,2}, Reagan Knight^{1,2}, Erin Vongchan^{1,2}, Samantha Schnabel^{1,2}, Bailey Hall^{1,2}, Kyra Sharp^{1,2}, Madison Geib^{1,2}, Abby Wingate^{1,2}, Alexander Bacanurschi^{1,2}, Ryan W. Naseman^{1,2}, Robert A. Lodder^{3,*}

University of Kentucky
Lexington, KY

1. Department of Pharmacy Services, University of Kentucky HealthCare, Lexington, KY 40536
2. Pharmacy Practice & Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536
3. Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536

*Author to whom correspondence should be addressed. Email: Lodder @ g.uky.edu
ORCID 0000-0001-6133-7561

RAPID COMMUNICATION

Abstract

Thyrotropin alfa is a recombinant heterodimeric glycoprotein that serves as a highly purified form of human thyroid stimulating hormone (TSH). It is produced through recombinant DNA technology within a genetically modified Chinese hamster ovary (CHO) cell line.

The molecule is structurally complex, consisting of two non-covalently linked subunits: an alpha subunit (92 amino acid residues) with two N-linked glycosylation sites, and a beta subunit (118 residues) containing one N-linked glycosylation site. The resulting amino acid sequence is identical to that of human pituitary TSH. Thyrotropin alfa is supplied as a sterile, non-pyrogenic, white to off-white lyophilized product, intended for intramuscular (IM) administration after reconstitution with Sterile Water for Injection, USP. Each vial of Thyrogen contains 1.1 mg thyrotropin alfa, 36 mg mannitol, 5.1 mg sodium phosphate, and 2.4 mg sodium chloride.

The intralot analysis of the 12 vials sampled from lot EY0155 revealed significant differences in the drug product within the same manufacturing lot, as evidenced by both spectral plots and multivariate analysis. The visual differences were observed in the following wavenumber regions: 4000 to 5000 cm^{-1} , 5150 cm^{-1} , and the 6700 cm^{-1} regions. The differences seen at 5150 cm^{-1} are very likely a difference in moisture content. 25% of the vials were flagged as outliers, with vials appearing 3.3, 3.8, and 5.3 SDs from the center of the cluster of 12 vials.

The interlot analysis of a spectral library containing 171 vials from 18 different lots of thyrotropin alfa demonstrated tremendous variability in the near-infrared absorption profiles across the samples. Obvious differences were observed in the spectra of the library at several wavenumber regions, including around 5900 and 6650 cm^{-1} . The ratio between two peak sizes between 5900 and 5950 cm^{-1} was dramatically variable. Differences were also noted around 4250, 4400, and 4540 cm^{-1} , and from 4700 to 4800 cm^{-1} . Only one vial was an outlier, appearing 7.8 SDs away from the center of the library spectral cluster. Some literature suggests differences in the spectra of vials may reflect differences in the stability and efficacy of the drug.

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

Thyrotropin alfa for injection (Thyrogen®, Sanofi Genzyme) is supplied as a sterile, non-pyrogenic, white to off-white lyophilized product, intended for intramuscular (IM) administration after reconstitution with Sterile Water for Injection, USP. Each vial of Thyrogen contains 1.1 mg thyrotropin alfa, 36 mg mannitol, 5.1 mg sodium phosphate, and 2.4 mg sodium chloride. After reconstitution with 1.2 mL of Sterile Water for Injection, USP, the thyrotropin alfa concentration is 0.9 mg/mL. The pH of the reconstituted solution is approximately 7.0 ([Isaacs, 2023a](#)). [Figure 1](#) is a photo of the drug product.



Figure 1. Photo of the drug product from lot EY0155. Spectra were collected through the bottom of the unopened vials.

The 18 lot numbers making up the 171 spectra in the spectral library were 0Y0272, BY0046, BY0122, BY0131, BY0349, BY0351, BY0484, CY0092, CY0379, DY0006, DY0019, DY0079, DY0365, DY0368, DY0467, EY0040, EY0100, and EY0155.

Background

The complexity of recombinant protein manufacturing, coupled with the critical nature of its clinical application, means that the global market for thyrotropin alfa is both highly regulated and

relatively concentrated. In the context of pharmaceutical economics and finance, drug quality problems—such as contamination, manufacturing deviations, or regulatory non-compliance—can have profound repercussions. These issues not only threaten patient safety but also have the potential to significantly impact the financial performance, share price, and overall market value of the companies involved. Data-driven analysis of how drug quality problems have affected the share price and value of companies manufacturing can have immediate and substantial impacts on company value, particularly for small companies in markets with limited competition and high regulatory scrutiny.

The Drug Quality Task Force at the University of Kentucky has previously reported on variability of thyrotropin alfa ([Isaacs, 2023b](#)), a drug used in the follow-up of well-differentiated thyroid cancer. The study used Fourier transform near-infrared (FTNIR) spectrometry to analyze 30 samples from four different lots, revealing inter-lot variability where the vials clustered into two distinct groups. Furthermore, one vial was identified as a significant outlier, suggesting it represents a different material, which highlights potential quality defects in the pharmaceutical supply chain.

A recent paper ([Cherepanov, 2025](#)) highlights the significant variability of thyroglobulin (Tg) structure, particularly concerning the presence of thyroiditogenic epitopes which are necessary to induce an autoimmune response like in Hashimoto's thyroiditis, which affects 1-5% of the population ([Bhattarai, 2022](#)). The study compares freshly isolated Tg, which carries these epitopes, with lyophilized Tg, which has lost them. This loss is associated with structural changes, including a weakening or disappearance of diiodotyrosine (DIT) oscillations and a decrease in the proportion of alpha-helices in the secondary structure. Furthermore, Tg isolated in the presence of the serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) is also shown to lose its native characteristics, exhibiting a low proportion of alpha helices and changes in iodinated tyrosine residues, suggesting this extraction method is not suitable for obtaining native, thyroiditogenic Tg. This indicates that the thyroiditogenic property of Tg is highly dependent on its structural integrity, particularly its iodination and secondary structure. FTIR spectroscopy can be used to detect thyroiditogenic epitopes on thyroglobulin.

While thyrotropin alfa is not known to initiate autoimmunity, high levels of native TSH (thyroid-stimulating hormone) have been theoretically linked to the perpetuation or exacerbation of an existing autoimmune response. This concept, sometimes called the "hypothyroid-autoimmune loop," suggests that elevated TSH levels (in a hypothyroid state) stimulate the remaining thyroid cells, which may in turn lead to an increased presentation of autoantigens (thyroglobulin and thyroid peroxidase), thereby potentially feeding the autoimmune destruction characteristic of Hashimoto's thyroiditis.

Since thyrotropin alfa acts as a super-physiological surge of TSH, it could acutely stimulate any remaining thyroid tissue. In a patient with pre-existing, subclinical autoimmunity, this could theoretically be a transient stimulus, but this short-term pharmacological use (typically two days) is mechanistically and clinically distinct from the chronic, progressive immune system attack that defines Hashimoto's thyroiditis. The temporary use of thyrotropin alfa is not generally considered a factor in triggering new Hashimoto's thyroiditis. The immune system attack in

Hashimoto's is a complex, progressive process involving autoantibodies against thyroid peroxidase (TPO) and thyroglobulin (Tg), and T-cell activation, driven by genetic and environmental factors ([Kaur, 2025](#)).

Based on searches of the U.S. Food and Drug Administration (FDA) and related sources for the time period since January 1, 2024, no drug recalls for the finished pharmaceutical product Thyrogen (thyrotropin alfa injection) have been reported.

Thyroid-related products and diagnostic assays containing or testing for TSH, of which thyrotropin alfa is a synthetic form, have been subject to recalls or regulatory action in 2024 and 2025. The Beckman Coulter Access TSH (3rd IS) Assay recall was initiated on August 28, 2024. The reason for the recall was that certain lots of the reagent packs may produce erroneously low TSH results, which could potentially lead to misdiagnosis or improper patient management in thyroid or pituitary disorders.

The FastPack TSH Calibrator Kit, a Class II Device, was recalled on August 26, 2024 because the calibrator card had an incorrect barcode, which could lead to a successful system calibration but cause the TSH results obtained to be higher than expected

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, 2023c](#)).

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](#) (OpenAI). AI can be used in a variety of ways, including to brainstorm, organize thoughts, develop arguments, and edit.

Drug Product Sourcing and Traceability

To investigate potential sources of inter-lot variability related to manufacturing location, the country of origin for a drug can be determined using published methods ([Noh, 2025](#)). Information is compiled using data from the NIH [Dailymed](#) database and the [U.S. Food and Drug Administration Drug Establishments Current Registration](#) database.

Results and Discussion

Intralot analysis

Spectra graphs of Lot EY0155

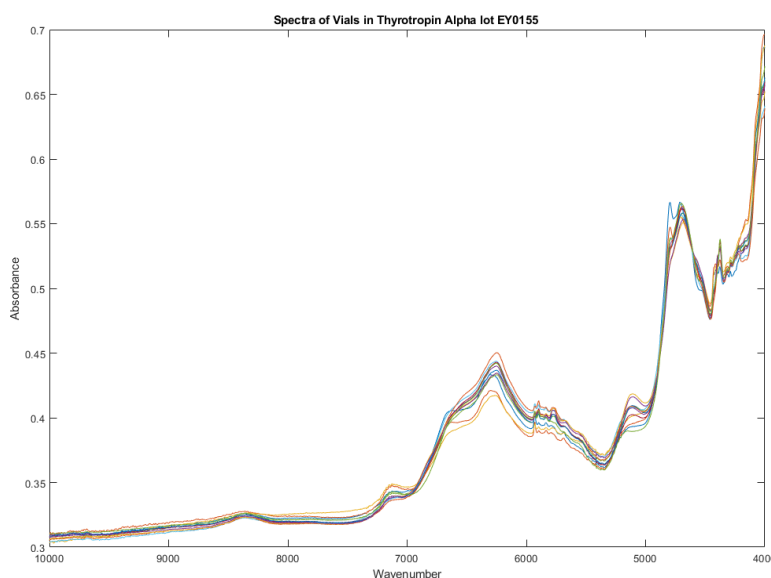


Figure 2. Spectra of the 12 files sampled from lot EY0155 show clear visual variability in the 4000 to 5000 cm^{-1} , 5150 cm^{-1} , and 6700 cm^{-1} regions. The differences seen at 5150 cm^{-1} is very likely a difference in moisture content.

[Figure 2](#) shows the FTNIR spectra for 12 different vials sampled from a single manufacturing lot, EY0155, of thyrotropin alfa. There is clear visual variability among the spectra of vials from within the *same lot*. Specifically, [Figure 2](#) points out variability in three regions: 4000 to 5000 cm^{-1} , 5150 cm^{-1} , and 6700 cm^{-1} . The differences observed at 5150 cm^{-1} are likely due to a difference in moisture content in the vials.

PC Plots

[Figure 3](#), which is a principal component (PC) plot of the 12 vials in lot EY0155, graphically illustrates the statistical variability within that single lot. Specifically, it identifies Vials 1, 3, and 6 as statistical outliers when analyzing the first three principal components (PCs 1-3). The multivariate analysis found these vials deviated from the center of the cluster by:

- **Vial 1:** 3.8 standard deviations (SDs)
- **Vial 3:** 5.3 standard deviations (SDs)
- **Vial 6:** 3.3 standard deviations (SDs)

This plot provides multivariate statistical evidence for the "significant differences in the drug product within the same manufacturing lot" that were first observed visually in the spectra from [Figure 2](#). Three of the 12 vials (25%) are more than 3 SDs from the center of all of the others, triggering the analysis of lot EY0155 along with the library of other lots recently scanned.

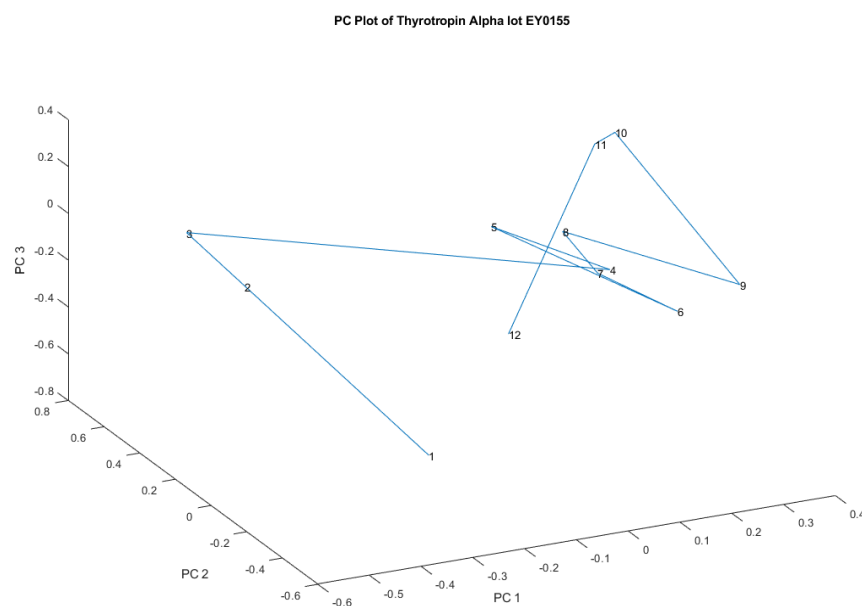


Figure 3. Analysis of the principal component scores plot for Lot EY0155 (n=12) identified Vials 1, 3, and 6 as statistical outliers (PCs 1-3). The observed deviations were 3.8, 5.3, and 3.3 standard deviations (SDs) for Vials 1, 3, and 6, in that order.

[Figure 4](#) shows exactly the same data as [Figure 3](#), but rotated to a different angle. It doesn't add new information but rather provides a different perspective on the 3D plot. The specific purpose of this rotation is to make it easier to see the direction in which vial 6 is displaced from the main cluster of samples.

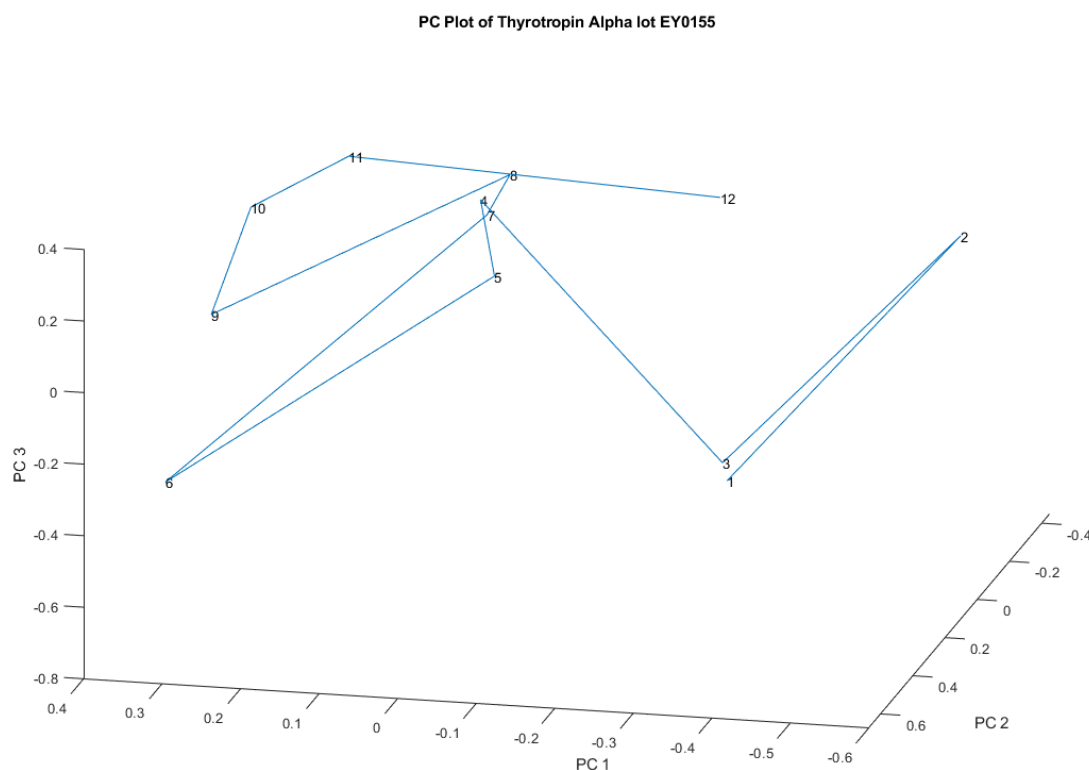


Figure 4. Another rotation of [Figure 3](#) shows the direction in which vial 6 is displaced from the center of the other samples.

[Table 1](#) quantifies the proportion of the total spectral variation that is captured by each principal component. The "Variation in this PC" column shows that the variation is not concentrated in a single component, but spread across several. For example, PC 1 accounts for the largest portion of the variance: 62.02%. The remaining components account for progressively smaller amounts of the variation.

It is possible to estimate which principal components are most important by looking at both the individual and cumulative variation values. The most important components are typically those with the highest "Variation in this PC" values. By this measure, PC 1, PC 2, and PC 3 are clearly the most important sources of spectral variation, as their values are substantially larger than those for PC 4 and beyond.

A common practice in principal component analysis is to determine how many components are needed to explain a target percentage of the total variance (e.g., 90% or 95%). The "Cumulative PC Variation" column shows:

To account for over 80% of the total variation the first two PCs (82.86%) must be used. To account for over 90% of the variation, the first three PCs, which together capture 91.65% of the total variation, must be used.

PC 1, PC 2, and PC 3 would typically be considered the most important because they are required to explain the vast majority (over 91%) of the spectral differences observed among the vials in lot EY0155. The analysis in [Figure 3](#), which identified outliers, was based on these three significant components.

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

PC Number	Variation in this PC	Cumulative PC Variation
1	0.6202	0.6202
2	0.2084	0.8286
3	0.0879	0.9165
4	0.0649	0.9814
5	0.0103	0.9917
6	0.0046	0.9963
7	0.0017	0.9979
8	0.0010	0.9989
9	0.0006	0.9995

Interlot analysis

Spectra of library

[Figure 5](#) displays the smoothed FTNIR spectra for the entire library of 171 vials of thyrotropin alfa, which were sampled from 18 different lots. This graph shows enormous variability in the near-infrared absorption profiles across all the samples. Specifically, there are obvious differences in the spectra around the 5900 cm^{-1} and 6650 cm^{-1} wavenumber regions. The ratio between two peak sizes in the 5900 cm^{-1} and 5950 cm^{-1} range is very variable, and in some samples, a new peak is noted as appearing as a "shoulder" at 6650 cm^{-1} .

The variations seen in [Figure 5](#) are not necessarily impurities but rather likely reflect variability in the molecular structure of the thyrotropin alfa glycoprotein itself. At a chemical and molecular level, this spectral variability is probably due to:

1. Post-Translational Modifications: The primary source of the spectral differences is probably the molecule's post-translational modifications (the chemical changes made to the protein after it is synthesized.)

2. Glycosylation Variants (Glycoforms): Thyrotropin alfa is a glycoprotein, meaning it has complex sugar chains attached to it. The drug is inherently produced as a complex mixture of glycosylation variants (glycoforms.) The spectrometric differences seen in [Figure 5](#) are the small, yet measurable, changes in the near-infrared absorption profile caused by this inherent, diverse population of glycoforms.
3. Sialylation Patterns: A key part of this heterogeneity is the distinct sialylation pattern. Sialylation refers to the number of terminal sialic acid residues on the molecule's sugar chains. The manufacturing process in Chinese hamster ovary (CHO) cells contributes to this heterogeneity, producing molecules that are sialylated (but not sulfated, unlike natural pituitary TSH).

This molecular variability can be clinically significant because distinct sialylation patterns affect the drug's stability and efficacy. The main role of sialylation is to increase the drug's stability and plasma half-life. A higher degree of sialylation makes the molecule more acidic. This acidic nature shields the glycoprotein from being recognized by liver receptors that clear it from the body. By evading this liver uptake, highly sialylated (more acidic) thyrotropin alfa has a significantly slower metabolic clearance rate. In summary, the spectral variability in [Figure 5](#) suggests that different vials and lots of the drug may contain different mixtures of glycoforms, which in turn could lead to differences in the drug's stability and how long it remains

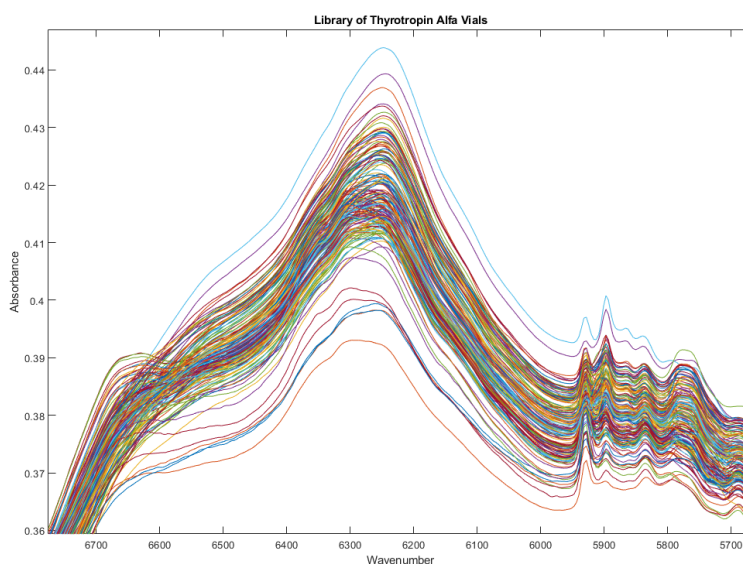


Figure 5. Spectra of the library of 171 vials collected over a 1000 cm^{-1} range. Obvious differences between the spectra are observed around 5900 and 6650 cm^{-1} . The ratio between the two peak sizes between 5900 and 5950 cm^{-1} is dramatically variable. In some samples another peak is feeding to appear as a shoulder at 6650 cm^{-1} .

active in a patient's body.

Using four spectra, [Figure 6](#) visually demonstrates the major spectral variations observed in the thyrotropin alfa library across a specific wavenumber range. The total number of spectra is reduced to make the differences between them easier to see. Changes in the peak ratios, especially in this spectral range (5700 to 6700 cm^{-1}), can be related to C-H and N-H stretching vibrations that reflect differences in the protein's secondary structure or the distribution of its glycoforms. By isolating these extreme examples from the principal component space, the figure clearly shows the structural heterogeneity that drives the large overall inter-lot variability detected in the thyrotropin alfa drug product.

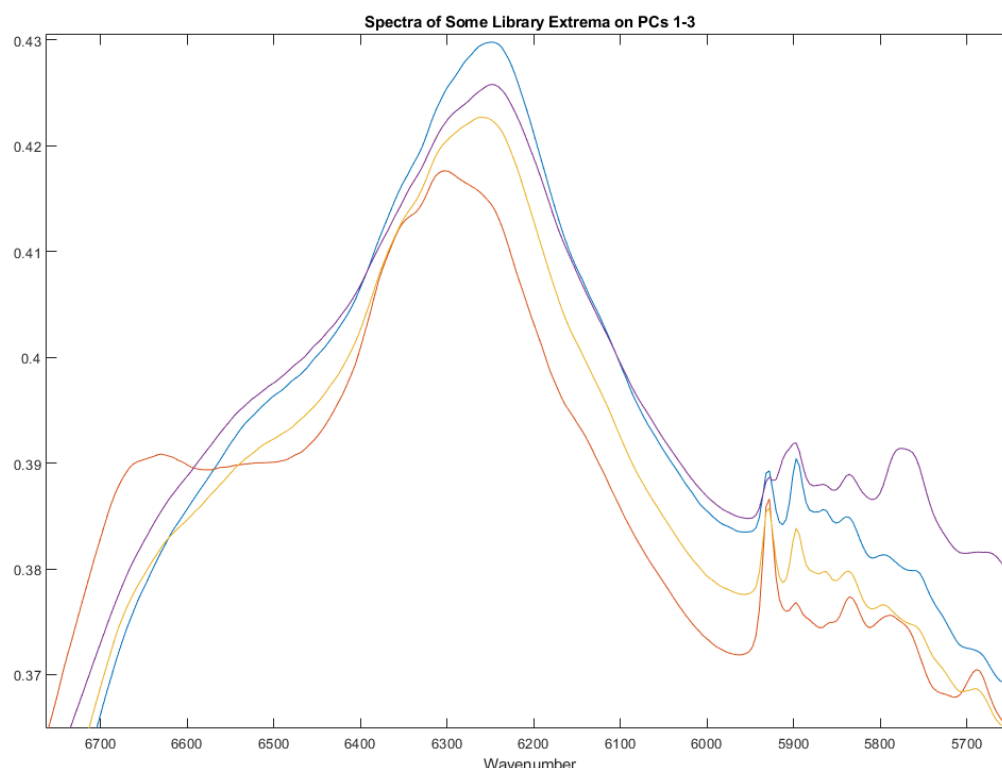


Figure 6. Four spectra selected from the library of spectra shown in [Figure 5](#). These four spectra represent the major variations that are observed in the region from 5700 to 6700 cm^{-1} . Note the differences in the peak ratios around 5900 cm^{-1} .

[Figure 7](#) shows the spectra of the library of the same 171 vials in [Figure 5](#), but in a different spectral range. In [Figure 7](#) the spectra were collected over a 700 cm^{-1} range. The figure illustrates obvious differences between the spectra across the various samples. These differences are specifically observed at the following wavenumber regions: 4220 cm^{-1} , 4400 cm^{-1} , 4540 cm^{-1} , and from 4700 to 4800 cm^{-1} . The spectral library displayed in [Figure 7](#) was used for the interlot analysis and contains 171 vials sourced from 18 different lots of thyrotropin alfa, demonstrating tremendous variability for a drug in near-infrared absorption profiles

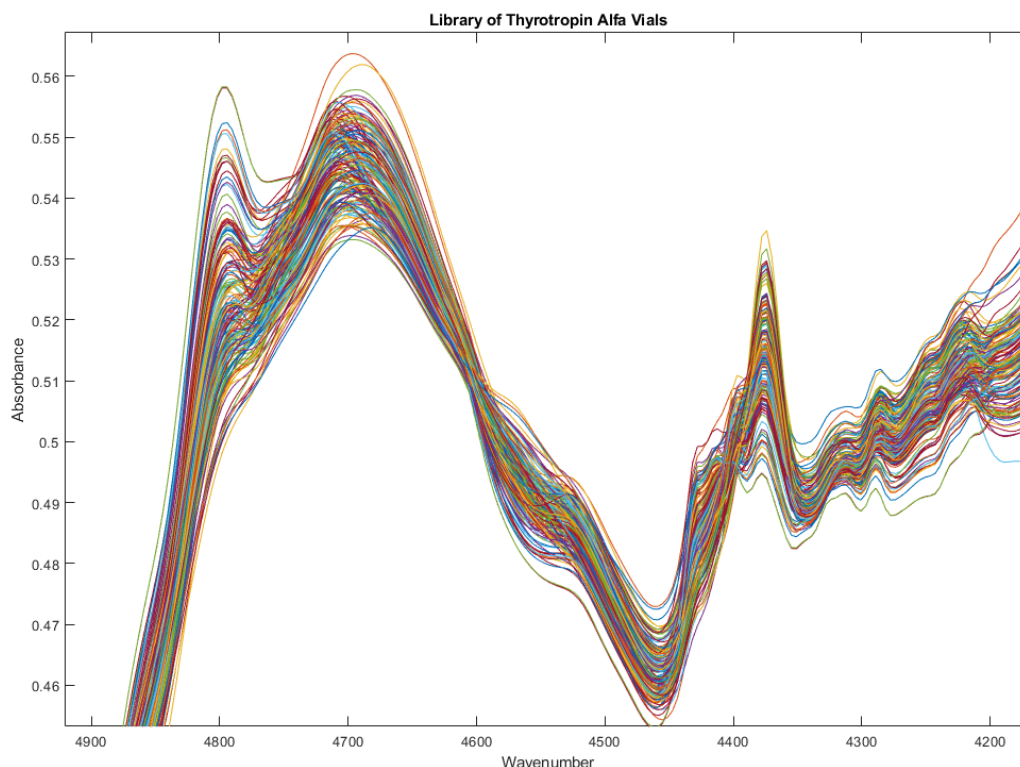


Figure 7. Spectra of the library of 171 vials collected over a 700 cm^{-1} range. Obvious differences between the spectra are observed at 4220 , 4400 , and 4540 cm^{-1} , and from 4700 to 4800 cm^{-1} .

[Figure 8](#) shows four spectra that were selected from the library of spectra shown in [Figure 7](#). These four selected spectra are intended to represent the major variations that are observed in the region from 4200 to 4800 cm^{-1} . The total number of spectra is reduced to make the differences between them easier to see. Plotting too many spectra on a single graph makes differences harder to see because it creates overplotting (the technical term for when data points and lines are plotted on top of each other, creating a dense, tangled mass), visual noise (the mass of overlapping lines acts as "visual noise" that drowns out the "signal", or the meaningful differences researchers seek). With visual noise, the brain has to work overtime just to process the jumble of lines, leaving little capacity to perform the actual task of comparison). [Figure 8](#) highlights the major variations within the specific wavenumber range from 4200 to 4700 cm^{-1} .

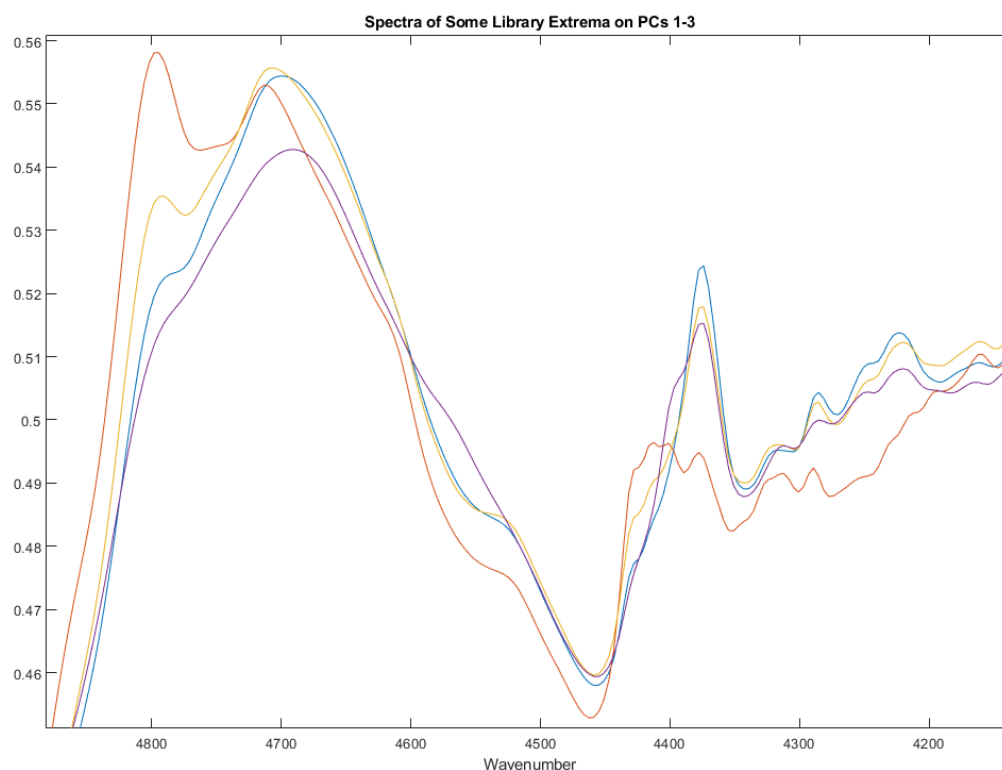


Figure 8. Four spectra selected from the library of spectra shown in [Figure 7](#). These four spectra represent the major variations that are observed in the region from 4200 to 4800 cm^{-1} .

[Figure 9](#) shows four spectra that were selected from the spectral library previously visualized in [Figure 5](#), [Figure 6](#), [Figure 7](#), and [Figure 8](#). The key feature of these specific spectra is that they appear to show varying levels of moisture content. [Figure 9](#) is important because it provides a visual representation of a specific source of variability identified in the thyrotropin alfa drug product. Moisture is a suspected variable. During the intralot analysis of lot EY0155, visual differences were observed in the spectral plots in the 5150 cm^{-1} region, which is very likely a difference in moisture content. The presence of varying moisture levels suggests that supervision of moisture content may be an area where the manufacturing process lacked sufficient control.

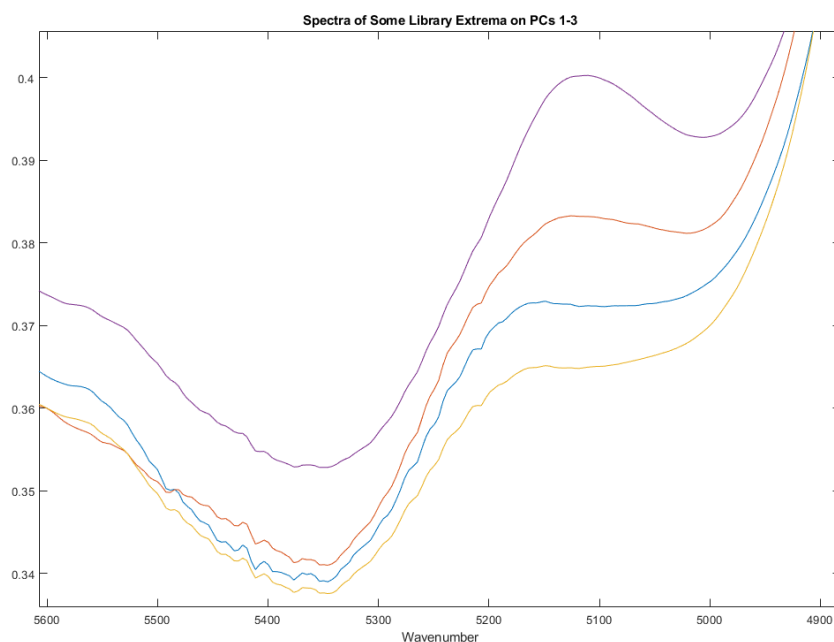


Figure 9. Four spectra that appear to show varying levels of moisture content, selected from the library of spectra shown in [Figure 5](#), [Figure 6](#), [Figure 7](#), and [Figure 8](#).

PC Plots

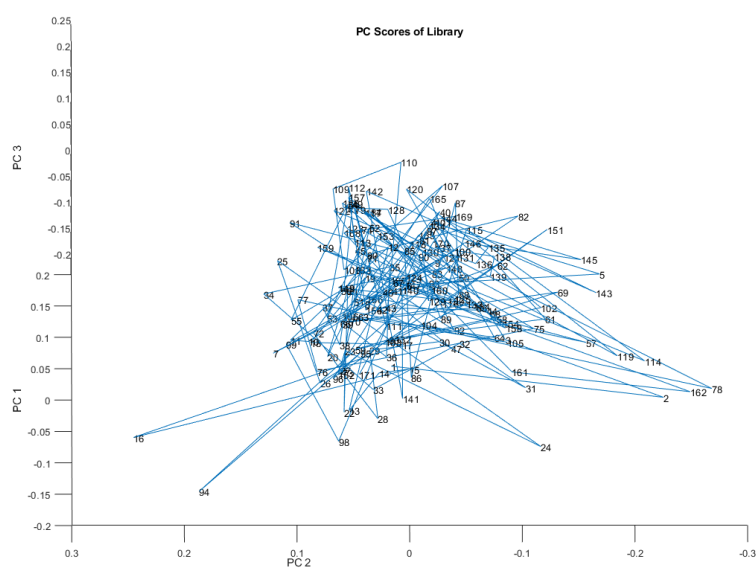


Figure 10. A scatter plot of principal component scores for the 171 samples in the spectral library. The tremendous variability of the vials means that no vials are statistically significant outliers on principal components 1, 2, and 3.

[Figure 10](#) shows a scatter plot of principal component scores for the 171 samples in the spectral library. No vials are statistically significant outliers on principal components 1, 2, and 3. The large variability in the spectra of the samples in the library mean that it's very difficult for one spectrum to be unusual, even if it looks very little like most of the other spectra in the library. The data represented in [Figure 10](#) contrasts with the standard expectation for pharmaceutical production data, where the first principal component (PC1) alone is typically expected to explain over 90% of the variance. For this spectral library, PC1 only accounts for 52.21% of the variation, demonstrating a higher degree of complexity which is reflected in the dispersed plot of PC scores.

In principal component analysis of random noise, each PC accounts for an amount of variation equal to that of the others. This happens because random noise has no structure or correlation. The entire purpose of PCA is to find the directions of maximum variance. PCA rotates the data to find a new set of axes (the Principal Components) that best describe the data's "shape."

Here's a breakdown using an analogy:

1. PCA on "Real" Data (A "Cigar")

Imagine the "real" spectral data (with signals) is a cloud of data points shaped like a long, thin cigar in 3D space. PCA's first job is to find the single axis that captures the most variance. It will immediately find the "long" axis of the cigar. This one component explains most of the data's shape. PCA then finds the next-best axis, which must be orthogonal (at a 90° angle) to PC1. This will be the "width" of the cigar. The final axis will be the "thickness" of the cigar, which accounts for less variance. In this case, the variance of $PC1 > PC2 > PC3$.

2. PCA on "Random Noise" (A Sphere)

Now, imagine random noise data. By definition, random noise means the variables are uncorrelated, and the variance is the same in all directions. This data cloud forms a perfectly uniform sphere. PCA tries to find the "long" axis of the sphere. But there isn't one. No matter which direction it chooses for PC1, the variance it captures is exactly the same as any other possible direction. The algorithm essentially "shrugs" and picks an arbitrary direction. PCA then tries to find the "next-best" axis, orthogonal to PC1. Since the data is a perfect sphere, any axis orthogonal to PC1 will capture the exact same amount of remaining variance. The same logic applies to the third axis. Because there is no inherent structure, correlation, or "long" direction to find, PCA cannot rank the components by importance. It simply divides the total random variance equally among the new, arbitrary axes it creates.

The data for thyrotropin alfa are somewhere in between a “long cigar,” where most of the variation is on the first PC, and random noise, where all of the PCs capture the same amount of variation.

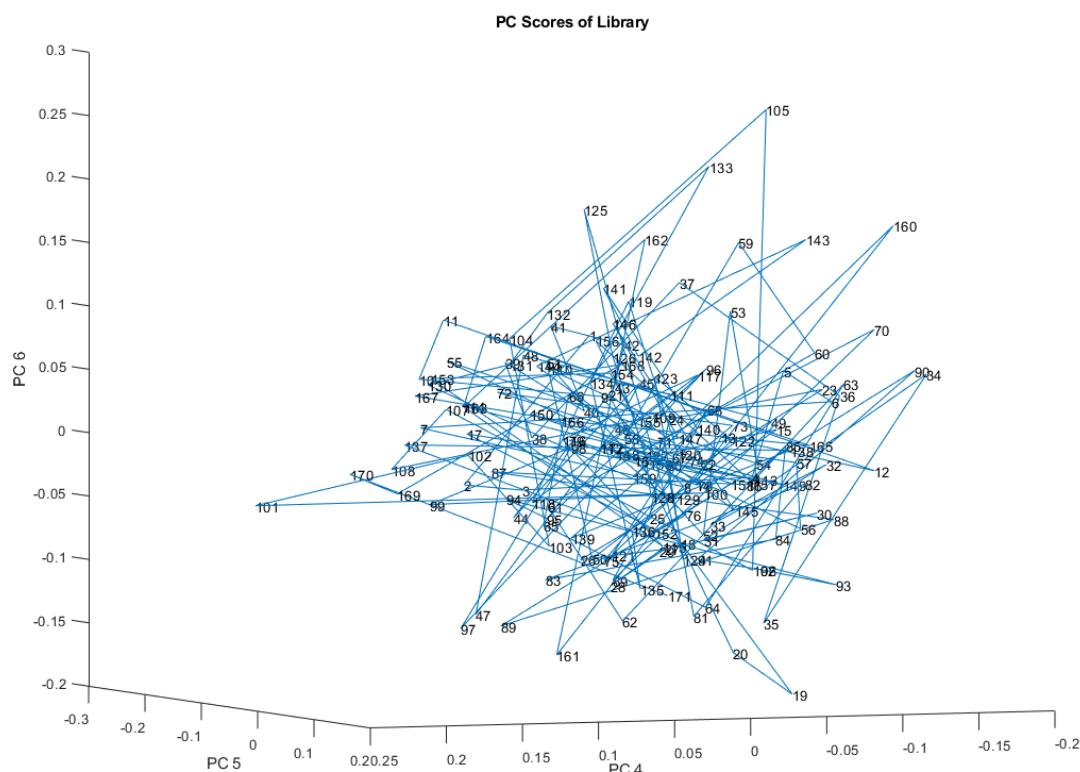


Figure 11. A scatter plot of principal component scores for the 171 samples in the spectral library. The huge variability of the vials means that no vials are statistically significant outliers on principal components 4, 5, and 6.

[Figure 11](#) is a scatter plot of principal component scores for the 171 samples in the spectral library. [Figure 11](#) specifically utilizes principal components (PCs) 4, 5, and 6. A key finding revealed by [Figure 11](#) is that because of the huge variability of the vials, no vials are statistically significant outliers when analyzed using principal components 4, 5, and 6, even when their spectra do not look very similar to many of the other spectra in the library.

[Figure 12](#) displays a scatter plot of principal component scores for the 171 samples in the spectral library. This plot uses the seventh, eighth, and ninth Principal Components (PCs 7, 8, and 9). The primary finding illustrated in this plot is the identification of a single sample, Vial 82, which is located 7.8 standard deviations (SDs) away from the center of the library spectral cluster, a definitive statistical anomaly.

[Figure 12](#) uses higher-order principal components (PCs 7, 8, and 9), which together account for only 0.96% of the total spectral variation (PC7: 0.44%, PC8: 0.32%, PC9: 0.20%). The displacement of vial 82 is primarily on PC 8 and PC 9. The fact that vial 82 is only detected as an outlier in this minor portion of the total variance means that its spectrum does not "appear much different from the others to the eye". This emphasizes the power of multivariate statistical analysis (like PCA) to detect subtle, yet significant, differences that would be missed by visual inspection of spectra.

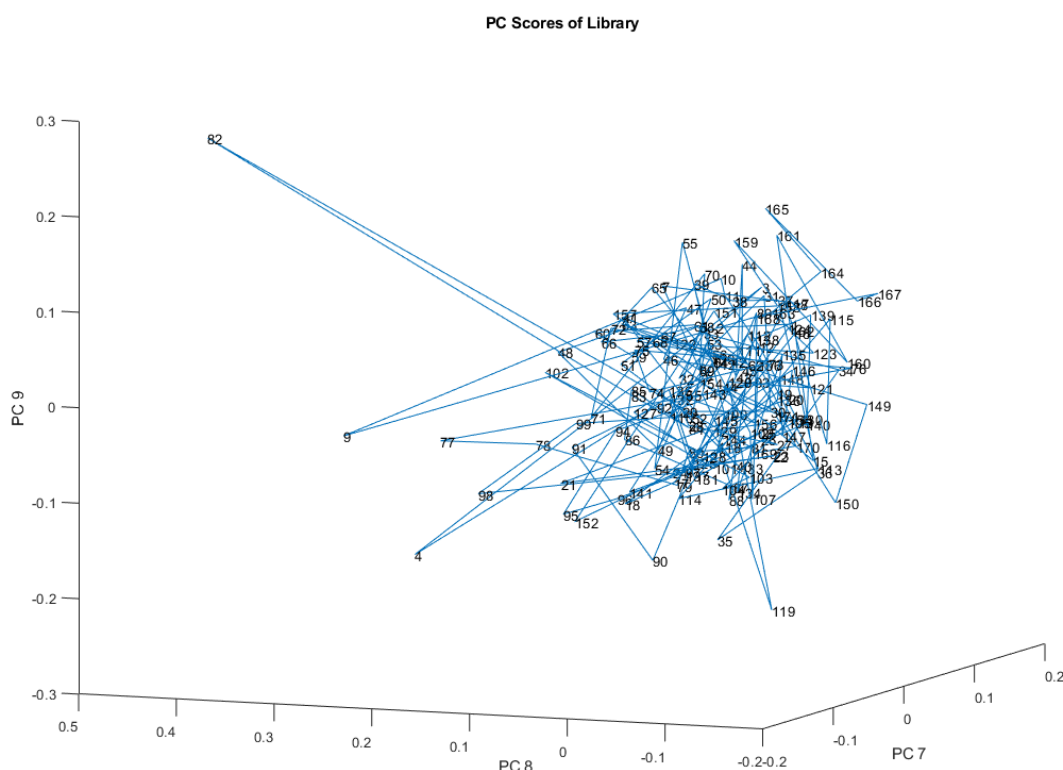


Figure 12. A scatter plot of principal component scores for the 171 samples in the spectral library. Using PCs 7, 8, and 9, Vial 82 is 7.8 SDs away from the center of the library spectral cluster. PCs 7-9 together account for only 0.96% of the total spectral variation, so the spectrum of vial 82 does not appear much different from the others to the eye.

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	0.5221	0.5221
2	0.2688	0.7909
3	0.1257	0.9166
4	0.0518	0.9684

5	0.0105	0.9790
6	0.0070	0.9860
7	0.0044	0.9904
8	0.0032	0.9935
9	0.0020	0.9956

[Table 2](#) provides a quantitative breakdown of the variability found within the thyrotropin alfa spectral library, which contains spectra from 171 vials across 18 different lots. [Table 2](#) is important because it demonstrates the high degree of complexity and structural heterogeneity present in the thyrotropin alfa drug product, which deviates significantly from typical quality standards for pharmaceuticals.

The standard expectation for spectral data from tightly controlled pharmaceutical production is that the first principal component (PC1) explains greater than 90% of the total observed variance. However, the spectral data for thyrotropin alfa displayed a more complex variance distribution, with PC1 only accounting for 52%. It was necessary to include the first three principal components (PC1-3) to surpass the cumulative 90% variance threshold.

This distribution of variance across multiple PCs is linked to the structural heterogeneity of the thyrotropin alfa molecule, which is inherently synthesized as a complex mixture of glycosylation variants (glycoforms). The inherent, diverse population of glycoforms and distinct sialylation pattern cause small, yet measurable, changes in the near-infrared absorption profile across samples, which is quantified by the values in [Table 2](#).

PC Loadings Plots

[Figure 13](#) shows a spectral plot of the loadings for the first principal component (PC1) of the thyrotropin spectral library. The plot specifically highlights interesting spectral variations at the following wave numbers: 4436, 4798, 5696, and 8337 cm^{-1} . Figure 13 is important because it identifies the specific spectral features and wavelength regions that account for the greatest amount of variability observed across the 171 vials of thyrotropin alfa in the spectral library. While the first principal component in near-infrared spectra is typically dominated by baseline variations, this is "not completely true" in the spectra of the PC 1 loadings shown in [Figure 13](#).

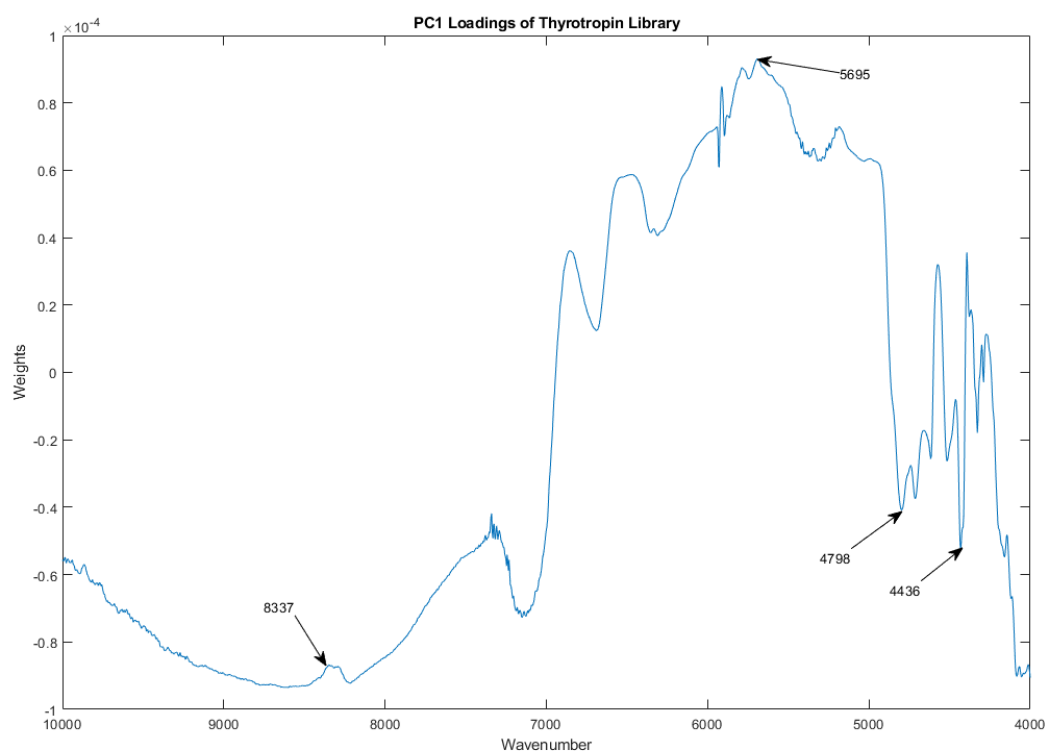


Figure 13. Spectral plot of the loadings for the first principal component of the thyrotropin spectral library. In near-infrared spectra, the first principal component is usually dominated by baseline variations. That is not completely true in the spectra of the PC1 loadings, which show interesting spectral variations at 4436, 4798, 5696, and 8337 cm^{-1} .

[Figure 14](#) displays a spectral plot of the loadings for the second principal component (PC2) of the thyrotropin alpha spectral library. [Figure 14](#) marks important spectral features at the following wave numbers: 4442, 4681, 5301, 6335, and 6961 cm^{-1} . Figure 14 quantifies the second largest source of spectral variation. The PCA used in the interlot analysis decomposes the total variation into orthogonal components. Table 2 shows that PC2 accounts for 0.2688 (26.88%) of the total spectral variation observed in the library.

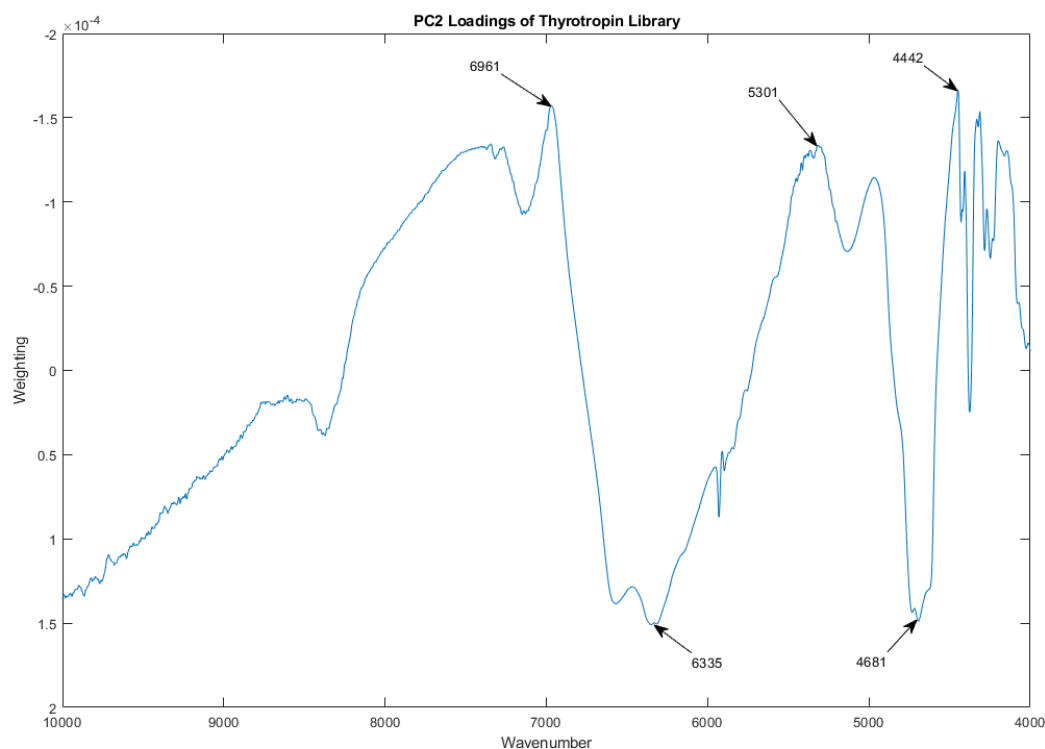


Figure 14. Spectral plot of the loadings for PC2 of the thyrotropin spectral library. Important spectral features at 4442, 4681, 5301, 6335, and 6961 are marked.

[Figure 15](#) shows a spectral plot of the loadings for the third principal component (PC3) of the thyrotropin spectral library. The figure marks important spectral features at the wave numbers 4376, 4423, 4849, and 6755 cm^{-1} .

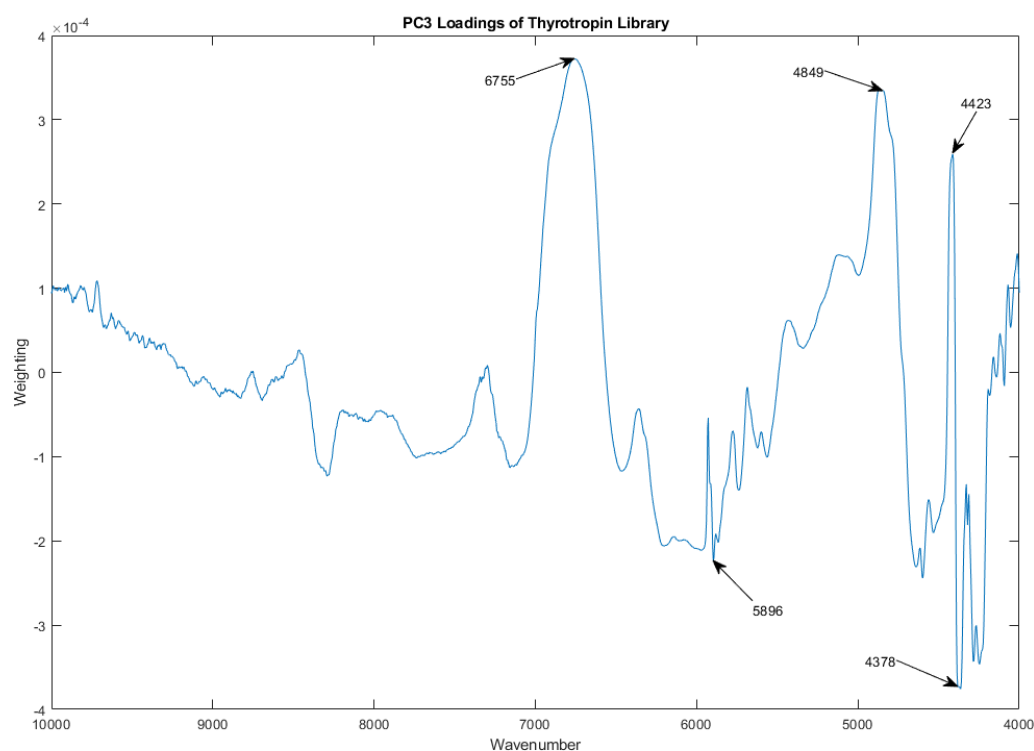


Figure 15. Spectral plot of the loadings for PC3 of the thyrotropin spectral library. Important spectral features at 4376, 4423, 4849, and 6755 cm^{-1} are marked with arrows.

[Figure 16](#) addresses the spectral variance analysis of the thyrotropin alfa drug product library, and displays a spectral plot of the loadings for the fourth principal component (PC4) of the thyrotropin spectral library. The figure highlights important spectral features at the following wave numbers: 4546, 4746, 5095, 5928, and 6864 cm^{-1} , which are marked with arrows. 4546 cm^{-1} could be a combination band of aldehyde C-H stretch and carbonyl C=O stretch, or combination bands originating from an aromatic ring (ring deformation and C-H stretching modes). The first overtones of O-H and N-H bonds generally occur at the higher wavenumber end of the NIR region (near 6864 cm^{-1}) due to their lower reduced mass and high anharmonicity. These features are sensitive to hydrogen bonding.

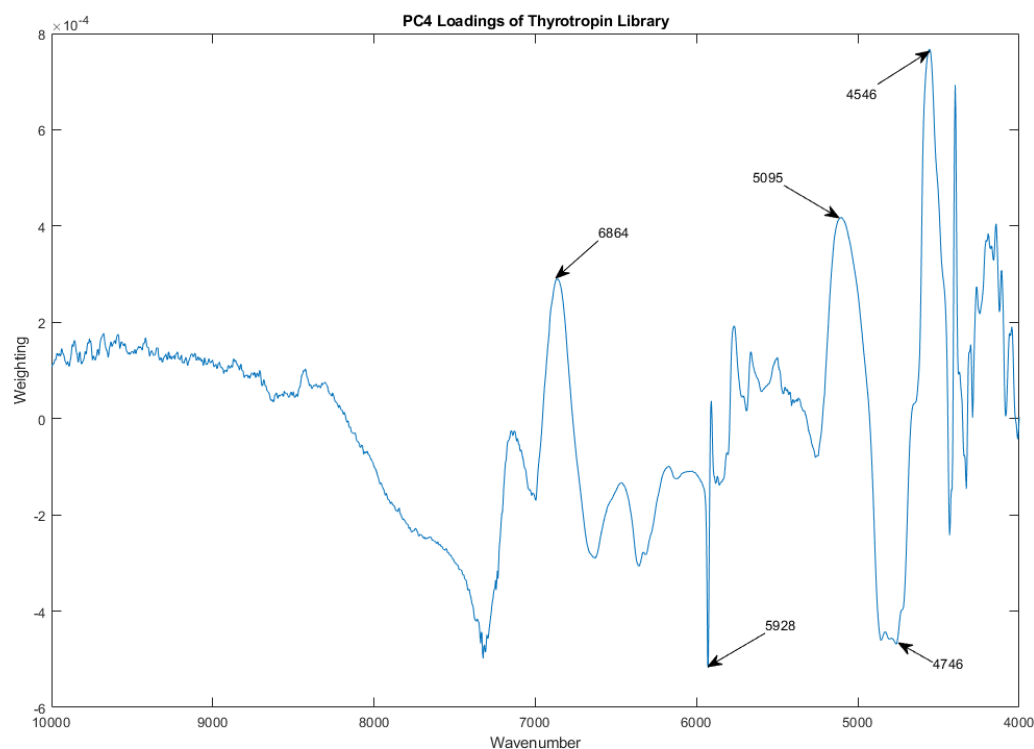


Figure 16. Spectral plot of the loadings for PC4 of the thyrotropin spectral library. Important spectral features at 4546, 4746, 5095, 5928, and 6864 cm^{-1} are marked with arrows.

[Figure 17](#) displays the specific wavenumbers that contribute most heavily to the variance represented by PC 5. Important spectral features on the plot are marked with arrows at 4520, 6632, 7103, and 8382 cm^{-1} . This plot is derived from the interlot analysis of the spectral library that contains 171 vials sampled from 18 different lots of thyrotropin alfa. While PC 5 accounts for a smaller amount of variance, its necessary inclusion highlights the complexity of the sample set. PC 5 accounts for 0.0105 (1.05%) of the total spectral variation, bringing the cumulative variance captured up to 97.90% when combined with PC 1 - PC 4. In the NIR region, absorption bands originate exclusively from non-fundamental transitions, such as overtones and combination bands. A peak at 4520 cm^{-1} is generally interpreted as a combination band, representing the sum of two underlying vibrational frequencies. Combination bands involving ring deformation modes and several other modes, mostly CH stretching modes, can appear in this spectral region.

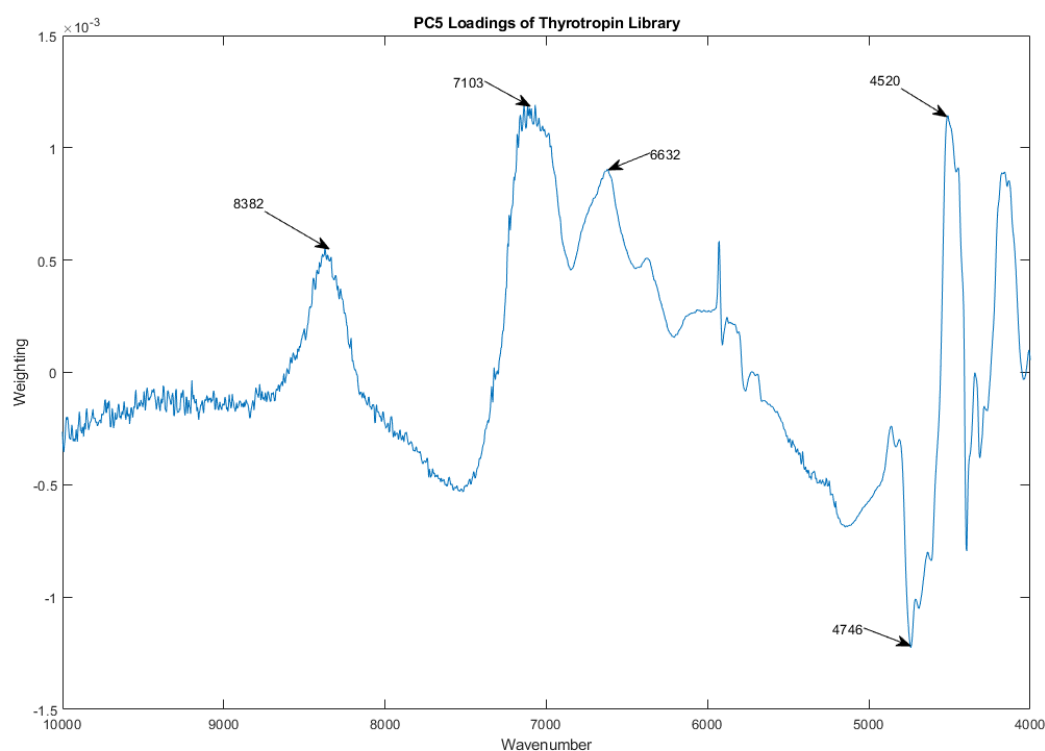


Figure 17. Spectral plot of the loadings for PC5 of the thyrotropin spectral library. Important spectral features at 4520, 6632, 7103, and 8382 cm^{-1} are marked with arrows.

[Figure 18](#) shows a spectral plot of the loadings for the sixth principal component (PC6) of the thyrotropin spectral library. This plot marks specific, important spectral features with arrows at the following wavenumbers: 4604, 4882, 5224, 5766, and 6864 cm^{-1} . The inherent, diverse population of thyrotropin alfa glycoforms (glycosylation variants) and distinct sialylation patterns cause small, but measurable, changes in the near-infrared absorption profile across samples. Analyzing the loadings plots for multiple PCs (like PC 6) helps reveal which specific spectral features (and thus which chemical or structural differences) are responsible for the variability detected during the interlot analysis.

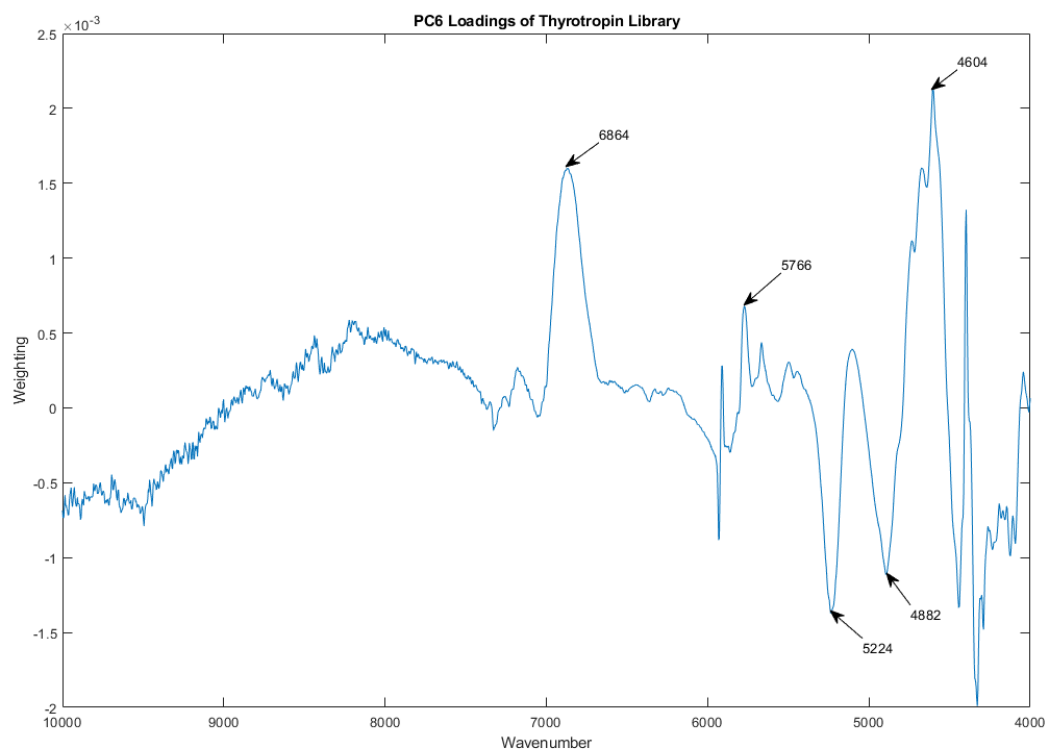


Figure 18. Spectral plot of the loadings for PC6 of the thyrotropin spectral library. Important spectral features at 4604, 4882, 5224, 5766, and 6864 cm^{-1} are marked with arrows.

[Figure 19](#) shows a spectral plot of the loadings for the seventh principal component (PC 7) of the thyrotropin spectral library. By PC 7 a considerable amount of spectroscopic noise is beginning to move in from the high wavelength end of the spectrum. This plot specifically marks important spectral features with arrows at the following wavenumbers: 4404, 5211, 5437, 5751, and 7032 cm^{-1} . PC 7, alongside PCs 8 and 9, was utilized to locate an unusual outlier. Using PCs 7, 8, and 9 (which together account for only 0.96% of the total spectral variation), Vial 82 was identified as being 7.8 standard deviations (SDs) away from the center of the library spectral cluster, suggesting a potential quality defect in that vial.

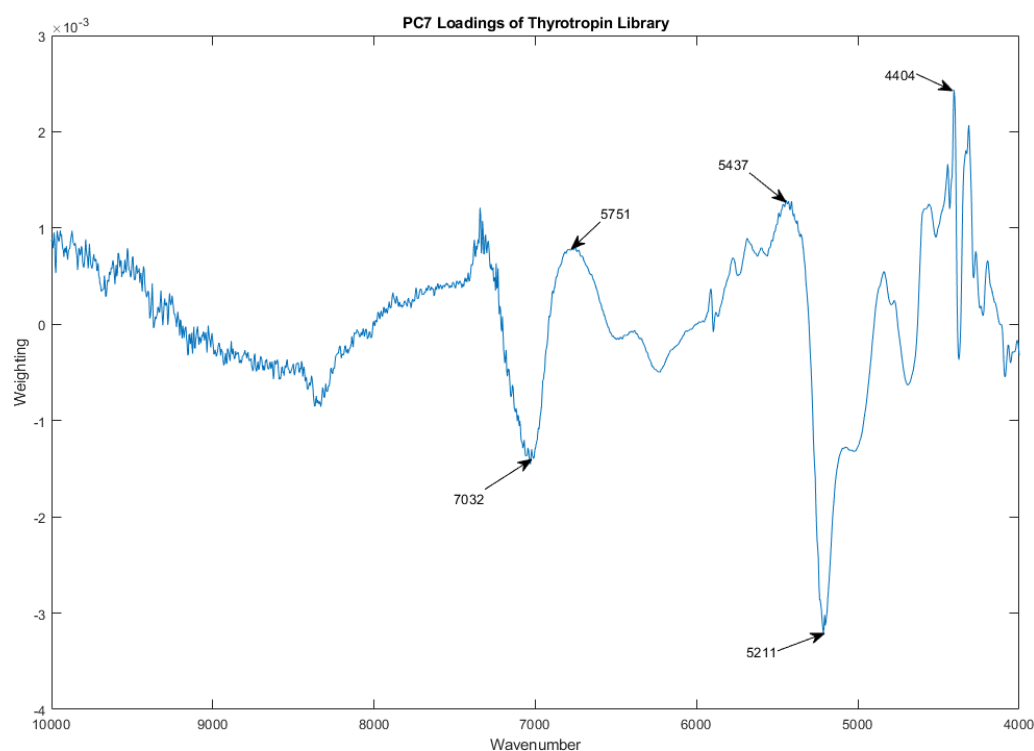


Figure 19. Spectral plot of the loadings for PC7 of the thyrotropin spectral library. Important spectral features at 4404, 5211, 5437, 5751, and 7032 cm^{-1} are marked with arrows.

[Figure 20](#) shows a spectral plot of the loadings for the eighth principal component (PC 8) of the thyrotropin spectral library. This plot marks specific, important spectral features with arrows at the following wavenumbers: 4352, 4681, 4785, 5205, and 6987 cm^{-1} . Even more noise has appeared at the high wavenumber end of the spectrum.

A peak observed at 6987 cm^{-1} (which corresponds to approximately 1431 nm) falls within the near-infrared region and is highly characteristic of the first overtone of stretching vibrations involving hydrogen atoms coupled with larger atoms, specifically O-H and N-H bonds. These overtones occur in the NIR because these vibrations are highly anharmonic due to the low reduced mass of the small hydrogen atoms, leading to stronger overtones.

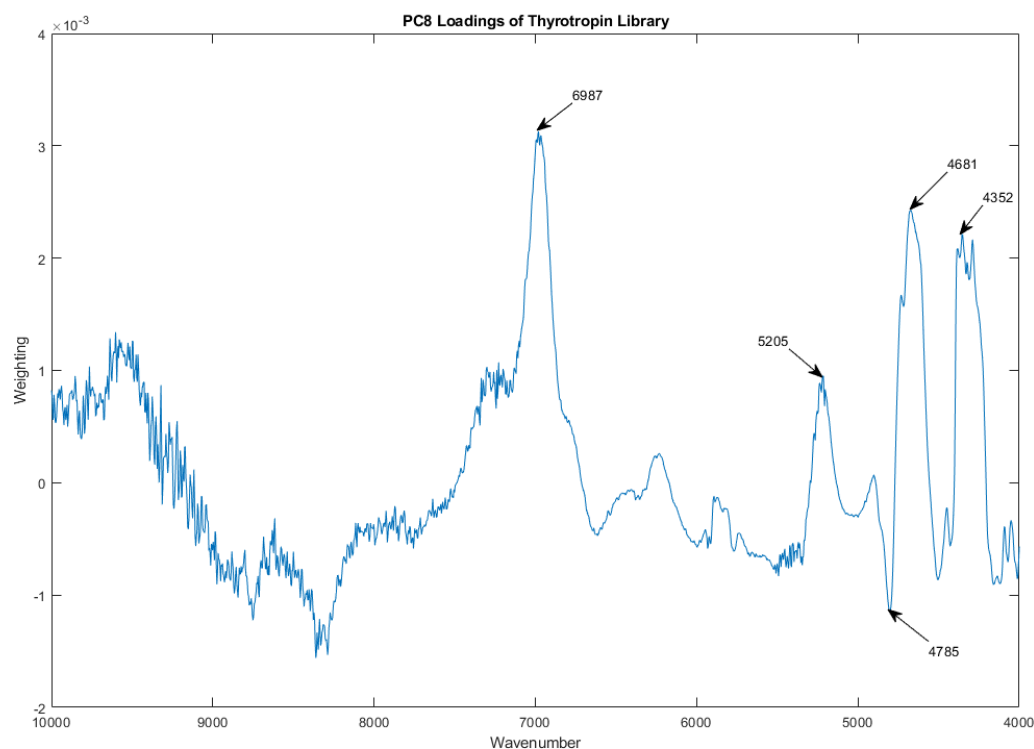


Figure 20. Spectral plot of the loadings for PC8 of the thyrotropin spectral library. Important spectral features at 4352, 4681, 4785, 5205, and 6987 cm^{-1} are marked with arrows.

[Figure 21](#) is noteworthy because it provides a visual representation of the specific spectral regions that contribute to the variation captured by the ninth principal component (PC 9) in the analysis of the thyrotropin spectral library. The left half of the loadings spectrum is now heavily contaminated with noise. The plot highlights five important spectral features (wavenumbers) that are marked with arrows: 4320, 4882, 6742, 7149, and 8473 cm^{-1} . Principal components are used to decompose the total variance of the spectral data. According to [Table 2](#), PC 9 accounts for 0.0020 of the variation in the spectra in the library. Although PC 9 contributes a minimal amount to the total variance, principal components are critical for revealing underlying structure in the data. When using Principal Components (PCs) 7, 8, and 9 together, one specific vial (vial 82) was identified as a significant outlier, appearing 7.8 SDs away from the center of the library spectral cluster. The low percentage of variance accounted for by these PCs (PCs 7-9 together account for only 0.96% of the total spectral variation) means the spectrum of vial 82 does not appear dramatically different to the naked eye. [Figure 21](#) helps pinpoint the subtle spectral variations captured by PC 9 that enable this statistical separation.

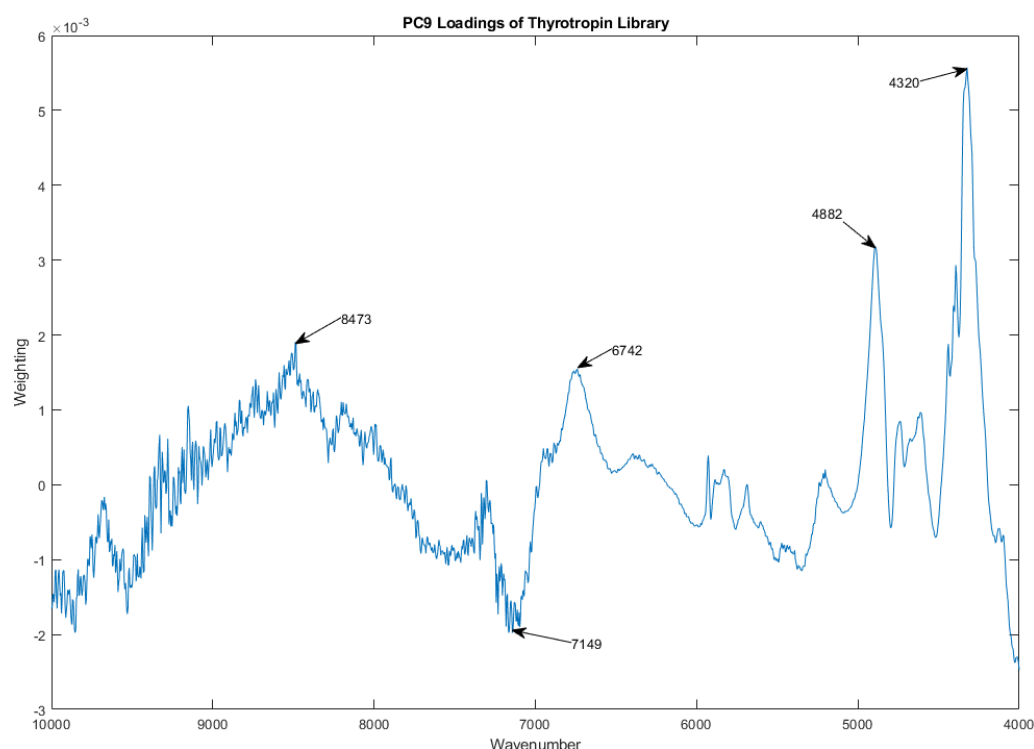


Figure 21. Spectral plot of the loadings for PC9 of the thyrotropin spectral library. Important spectral features at 4320, 4882, 6742, 7149, and 8473 cm^{-1} are marked with arrows.

Tracing Manufacturing Location

Tracing the origins of the thyrotropin alfa revealed that the drug product was sourced from multiple manufacturing locations (see [Table 3.](#)) 'ANALYSIS' is the most common operation, occurring 18 times. 'MANUFACTURE' is the second most common, with 14 occurrences. 'API MANUFACTURE' is also significant, with 11 occurrences. Less frequent operations include 'LABEL', 'PACK', 'RELABEL', and 'REPACK' (each with 4 occurrences), and 'STERILIZE' (with 2 occurrences). Firms like 'Genzyme Corporation' perform a wide range of operations including 'ANALYSIS', 'API MANUFACTURE', 'LABEL', 'MANUFACTURE', 'PACK', 'RELABEL', and 'REPACK'.

Other firms such as 'Ablynx', 'Genzyme Flanders', and 'SANOFI WINTHROP INDUSTRIE' primarily focus on 'ANALYSIS' and 'MANUFACTURE', with 'Genzyme Flanders' and 'SANOFI WINTHROP INDUSTRIE' also involved in 'API MANUFACTURE'.

While the FDA maintains that drugs approved for the U.S. market must meet the same quality standards regardless of manufacturing location, and the agency conducts inspections of foreign facilities, differences in manufacturing environments or processes between sites could be a contributing factor to the observed interlot variability. This observation warrants further investigation to determine if specific manufacturing sites are associated with distinct spectral profiles, which could, in turn, have implications for product consistency and efficacy.

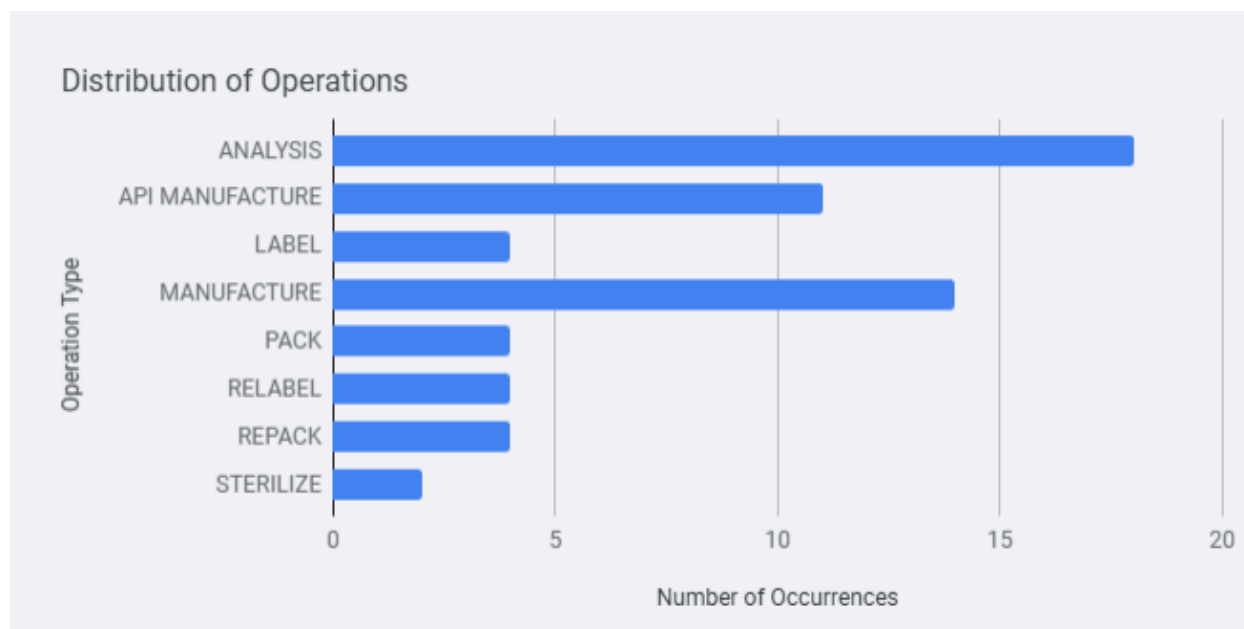


Figure 22. The most frequently observed operations are 'ANALYSIS' and 'MANUFACTURE'. 'ANALYSIS' is the most common operation, occurring 18 times. 'MANUFACTURE' is the second most common, with 14 occurrences. 'API MANUFACTURE' is also significant, with 11 occurrences.

Table 3. Facility locations and operations for thyrotropin alfa.

FEI NUMBER	DUNS NUMBER	FIRM NAME	ADDRESS	OPERATIONS
1220423	034378252	Genzyme Corporation	80 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	943130096	Genzyme Corporation	76 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	117450412	Genzyme Corporation	8 New York Avenue, Framingham, Massachusetts (MA) 01701-8806, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	02753675	Genzyme	78 New York Avenue, Framingham,	ANALYSIS; API

	6	Corporation	Massachusetts (MA) 01701, United States (USA)	MANUFACTURE; MANUFACTURE
1220423	96827891 6	Genzyme Corporation	45 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	96827893 2	Genzyme Corporation	74 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	96827887 4	Genzyme Corporation	51 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
1220423	96830265 8	Genzyme Corporation	68 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS
300362383 9	37215389 5	Genzyme Flanders	Cipalstraat 8, Geel, 2440, Belgium (BEL)	ANALYSIS; API MANUFACTURE; MANUFACTURE
300380984 0	98512741 9	Genzyme Ireland Limited	Unit 701 IDA Industrial Park, Old Kilmeaden Road, Waterford, X91 TP27, Ireland (IRL)	ANALYSIS; LABEL; MANUFACTURE; PACK; RELABEL; REPACK; STERILIZE
300891529 7	37200009 6	Ablynx	Technologiepark-Zwijnaarde 21 Gent,, ZWIJNAARDE, 9052, Belgium (BEL)	ANALYSIS; MANUFACTURE
300919403 3	28006572 4	SANOFI WINTHROP INDUSTRIE	23 boulevard Chambaud de la Bruyere, Lyon, 69007, France (FRA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
300938994 0	05042439 5	Genzyme Corporation	11 Forbes Road, Northborough, Massachusetts (MA) 01532, United States (USA)	ANALYSIS; LABEL; PACK; RELABEL; REPACK
1220423	96827887 4	Genzyme Corporation	51 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS; API MANUFACTURE; MANUFACTURE
300380984 0	98512741 9	Genzyme Ireland Limited	Unit 701 IDA Industrial Park, Old Kilmeaden Road, Waterford, X91 TP27, Ireland (IRL)	ANALYSIS; LABEL; MANUFACTURE; PACK; RELABEL; REPACK; STERILIZE
300938994 0	05042439 5	Genzyme Corporation	11 Forbes Road, Northborough, Massachusetts (MA) 01532, United States (USA)	ANALYSIS; LABEL; PACK; RELABEL; REPACK
1220423	96827891 6	Genzyme Corporation	45 New York Avenue, Framingham, Massachusetts (MA) 01701, United States	ANALYSIS; API MANUFACTURE;

			(USA)	MANUFACTURE
1220423	96830265 8	Genzyme Corporation	68 New York Avenue, Framingham, Massachusetts (MA) 01701, United States (USA)	ANALYSIS

Conclusion

Thyrotropin alfa (Thyrogen®) is a recombinant heterodimeric glycoprotein that serves as a highly purified form of human thyroid stimulating hormone (TSH). It is produced through recombinant DNA technology within a genetically modified Chinese hamster ovary (CHO) cell line.

The molecule is structurally complex, consisting of two non-covalently linked subunits: an alpha subunit (92 amino acid residues) with two N-linked glycosylation sites, and a beta subunit (118 residues) containing one N-linked glycosylation site. The resulting amino acid sequence is identical to that of human pituitary TSH.

The primary source of the observed spectral variability appears to lie in the molecule's post-translational modifications. Both native pituitary TSH and recombinant thyrotropin alfa are inherently synthesized as a complex mixture of glycosylation variants (glycoforms). The CHO expression system further contributes to this heterogeneity; while the resultant thyrotropin alfa is sialylated, it lacks the sulfation characteristic of the naturally occurring pituitary TSH.

This inherent, diverse population of glycoforms, along with the distinct sialylation pattern, causes small, yet measurable, changes in the near-infrared absorption profile across samples. This structural heterogeneity dictates that the total spectral variance is distributed across multiple orthogonal factors, thereby requiring the inclusion of up to three principal components (PC1-3) to adequately capture over 90% of the cumulative variation, a higher degree of complexity than typically observed for simple small-molecule or single-component large-molecule pharmaceuticals.

Distinct sialylation patterns of thyrotropin alfa can affect both its stability and efficacy. The degree of sialylation—the number of terminal sialic acid residues on the molecule's glycan chains—creates a crucial trade-off between its intrinsic activity and its lifespan in the body. The primary role of sialylation for thyrotropin alfa is to enhance its stability in the bloodstream, which is measured by its plasma half-life. A higher degree of sialylation makes the molecule more acidic. This acidic nature shields the glycoprotein from being recognized by specific receptors in the liver (like the asialoglycoprotein receptor) that are responsible for clearing it from the circulation ([Szkudlinski, 1993](#)). By evading this liver uptake, highly sialylated thyrotropin alfa has a significantly slower metabolic clearance rate (MCR) in the liver ([Zhu, 2024](#)).

The intralot analysis of the 12 vials sampled from lot EY0155 revealed significant differences in the drug product within the same manufacturing lot, as evidenced by both spectral plots and

multivariate analysis. The visual differences were observed in the following wavenumber regions: 4000 to 5000 cm^{-1} , 5150 cm^{-1} , and the 6700 cm^{-1} regions. The differences seen at 5150 cm^{-1} are very likely a difference in moisture content. 25% of the vials were flagged as outliers, with vials appearing 3.3, 3.8, and 5.3 SDs from the center of the cluster of 12 vials.

The interlot analysis of a spectral library containing 171 vials from 18 different lots of thyrotropin alfa demonstrated tremendous variability in the near-infrared absorption profiles across the samples. Obvious differences were observed in the spectra of the library at several wavenumber regions, including around 5900 and 6650 cm^{-1} . The ratio between two peak sizes between 5900 and 5950 cm^{-1} was dramatically variable. Differences were also noted around 4250, 4400, and 4540 cm^{-1} , and from 4700 to 4800 cm^{-1} . Only one vial was an outlier, appearing 7.8 SDs away from the center of the library spectral cluster.

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.

Acknowledgements

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