Levothyroxine Variations by Process Analytical Technology

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RAPID COMMUNICATION

Abstract

Intra-lot and inter-lot variability in the spectra of levothyroxine was detected in the Drug Quality Study (DQS) using Fourier transform near-infrared spectrometry (FTNIR). Two vials of 12 vials sampled from Athenex lot AFN102 appeared 10.1 and 9.1 SDs from the center of the rest of the vials on the DQS FTNIR screening assay. Spectra of 108 vials from six lots in the library clustered in two groups (p=0.02), suggesting they represent different material.

Introduction

The University of Kentucky's (UK) Drug Quality Study was established in August of 2019 to engage in consumer-level quality assurance testing for drugs used within UK HealthCare's pharmacies. DQS currently screens medications, using FTNIR and Raman spectroscopy, for quality defects indicated by variability in absorbance peak intensities and locations. Through 16 months of continuous monitoring, DQS has assembled a spectral library containing medications typically used in an inpatient care setting. Statistical analyses using DQS' spectral library can now be performed to identify potential intra-lot and inter-lot variability in medications under review. Using MedWatch, DQS reports its findings in an effort to hold manufacturers accountable for GMP requirements and to improve patient outcomes by exerting positive pressure on the pharmaceutical supply chain. At all levels, DQS staff are committed to achieving service excellence by pursuing compliance with the standards set forth by our patients and broad GxP requirements.

Drug Product

Levothyroxine Sodium for Injection is indicated for the treatment of myxedema coma or severe hypothyroidism (Ono, 2017) Off-label use of levothyroxine includes cadaveric organ recovery (Salim, 2001), where it is employed in brain-dead potential organ donors to reverse hemodynamic instability and to prevent cardiovascular collapse, leading to more available organs for transplantation. Since levothyroxine is considered a narrow therapeutic index drug (Burns, 1999), patients receiving therapy with this medication are at risk for clinically significant differences in therapeutic effect if they are underdosed or overdosed.

Issues

The DQS team has identified possible quality control issues (intra-lot and inter-lot variability) with Levothyroxine Sodium 100 µg manufactured by Athenex that may require further investigation.

The lot with intra-lot variability was lot AFN102 with an expiration date of 05/31/2023. The lots forming the spectral library were lots AFN001, AFN101, AFN102, AFN103, AFN903, and AFN904.

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected for inventory belonging to Lot AFN102 as part of routine medication quality screening. A representative sample of 12 individual vials were selected for screening from Lot AFN102 and noted to be stored under proper conditions, in their original packaging at ambient room temperature. FTNIR spectra were collected noninvasively and nondestructively through the bottom of the vials using a Thermo Scientific Antaris II FTNIR Analyzer (Waltham, MA, USA).

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 (Joliffe, 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 dusters 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 (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.

Results and Discussion

Intralot Analysis

Upon examination of the FTNIR spectra, significant absorbance peak location and intensity differences were observed in 2 of the 12 vials (vials 2 and 4 in Figures 1-3) screened after applying multiplicative signal correction to the spectra. In general, differences in absorbance peaks were observed at 4518, 4693, 6242, and 6540 cm^{-1}. These findings can be observed in Figures 1, 2, and 3.

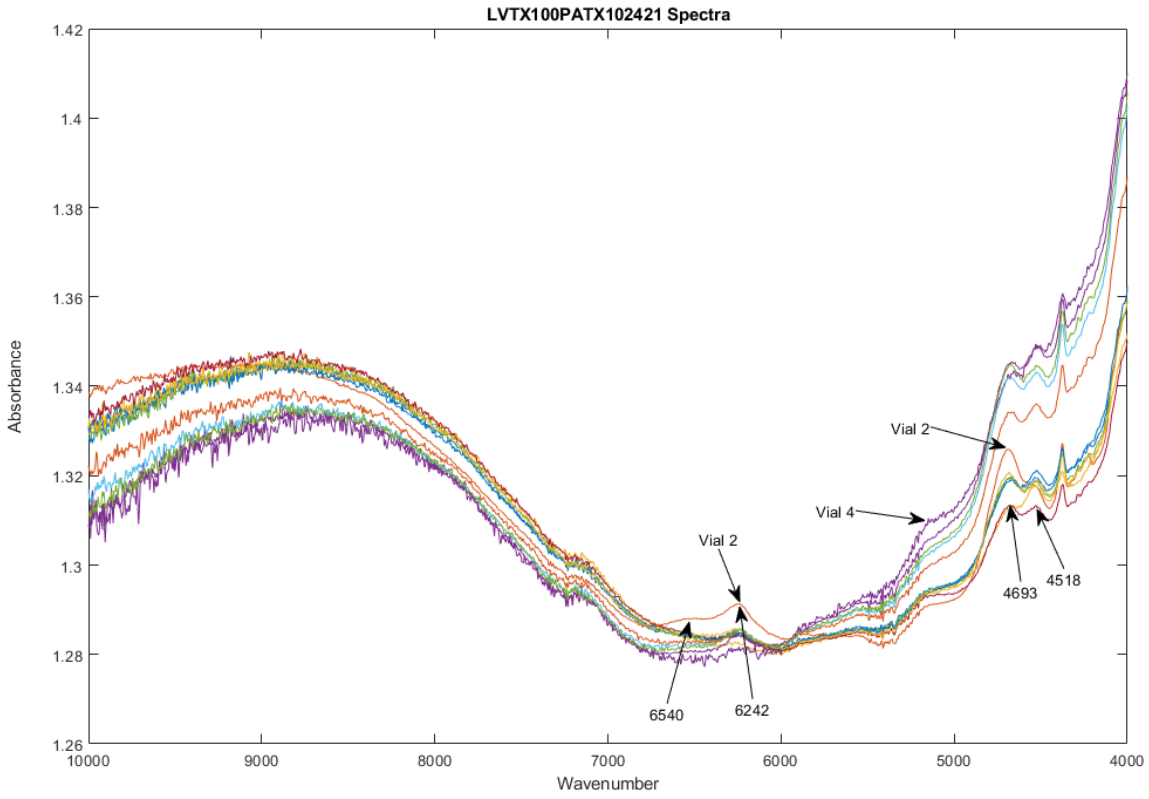

Figure 1. Spectra of 12 vials of levothyroxine from Lot AFN102. Two vials (numbers 2 and 4) are flagged as unusual, due in part to the changes in relative intensity of the signals at 4518 and 4693 cm^{-1}. A zoom-in on this region is provided in Figure 4. The appearance of more noise than usual in the spectra is due to the comparatively low amount of drug in the vials (100 µg).

Spectral variations in the vials in lot AFN102 include changes that appear to correspond to the amount of drug in the vials, the moisture content, and differences in chemical composition. Note in Figure 1 the ratios of relative intensities of the peaks at 4693 and 4518 cm^{-1}. There was no

obvious visual difference between any of the vials sampled from lot AFN102. Absorbance was collected in the spectra as the logarithm of the reciprocal reflectance (log(1/R)).

Figure 2 is a principal component plot of the scores of each of the 12 vials sampled from lot AFN102. Ten of the spectra cluster rather closely together, while two are outliers (vials 2 and 4, which plot as 10.1 and 9.1 SDs from the center of the cluster, respectively). Vial 4 does not appear as an outlier in the particular rotation shown in Figure 2, but it does look like an outlier in Figure 3, which is another rotation of the axes.

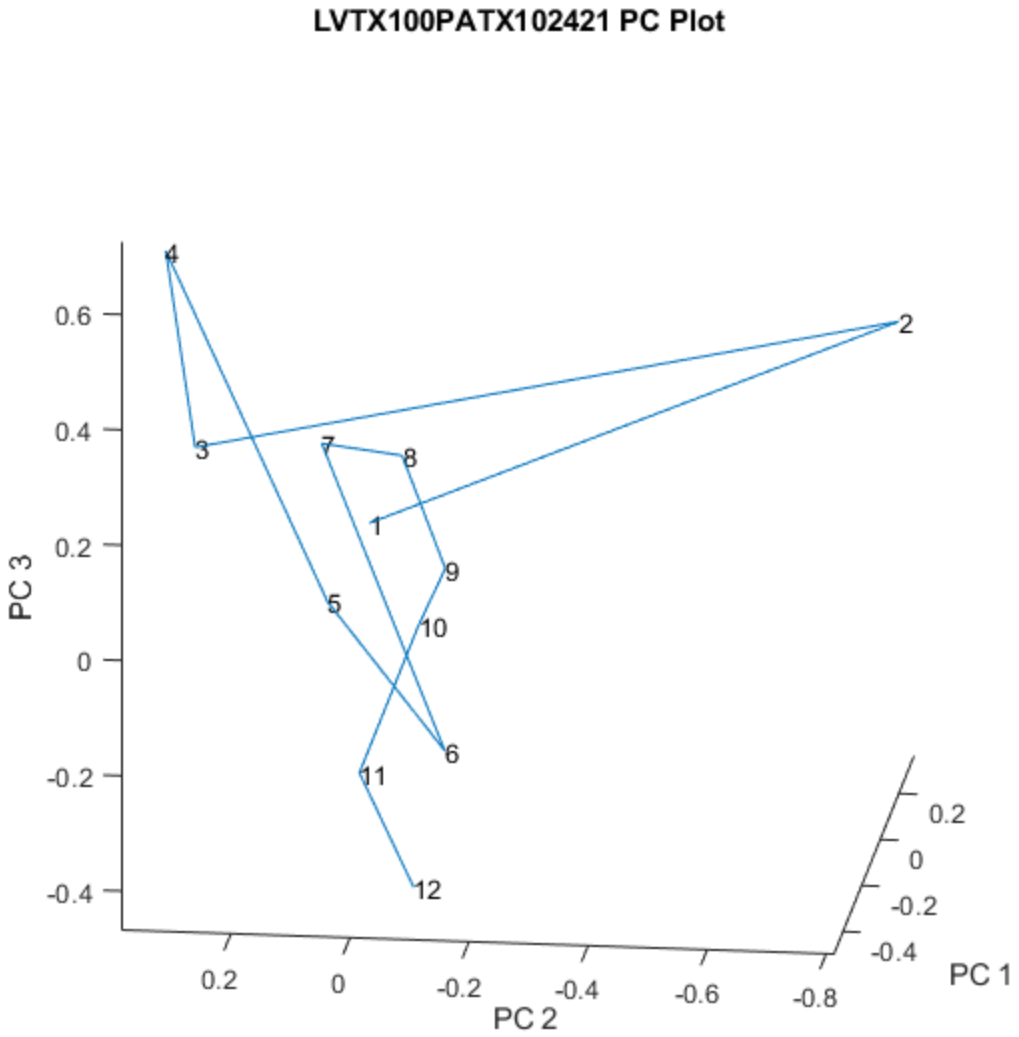

Figure 2. Principal components of spectra of 12 vials of levothyroxine from Lot AFN102. Two vials (numbers 2 and 4) are flagged as unusual, but vial 2 sticks out more than vial 4 in this view. Rotation of the axes produces Figure 3, in which both vials 2 and 4 appear as outliers (10.1 and 9.1 SDs from the center of the cluster, respectively).

Principal components 1-3 account for 84% of the spectral variation in the 12 vials sampled from lot AFN102. PC 1 is 58% of the variation on its own.

Principal components 1-3 together account for 52% of the total spectral variation of the library of 108 samples. PC 1 is 33% of the variation on its own in the spectral library.

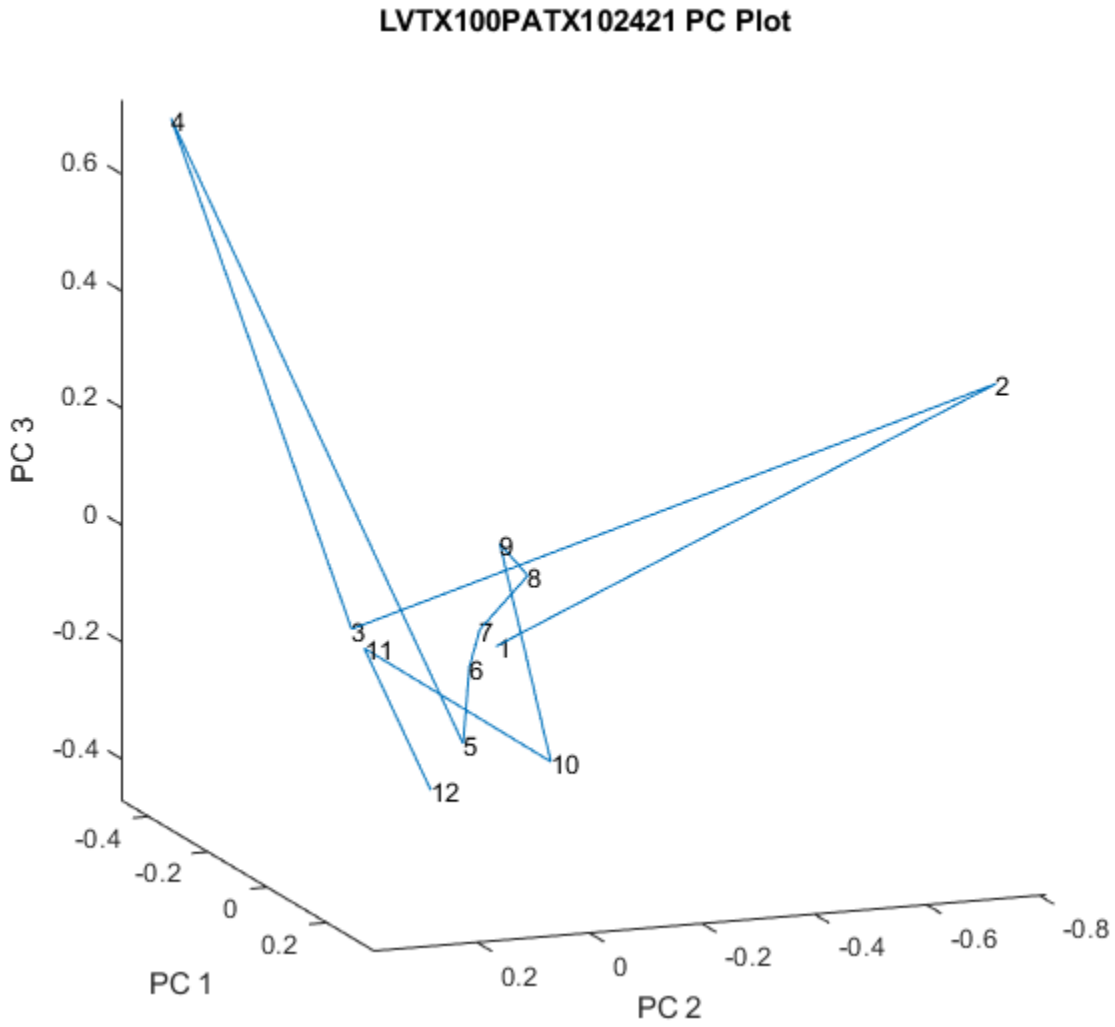

Figure 3. Principal components of spectra of 12 vials of levothyroxine from Lot AFN102. In this rotation, both vials 2 (10.1 SDs from center) and 4 (9.1 SDs from center) appear to be outliers.

The observation of variability in absorbance peak intensity and absorbance peak location differences for vials within the lot may indicate the presence of product impurities and/or degradation products in addition to variability in the amount of drug present in each vial.. Statistical analysis of the collected FTNIR spectra suggest intra-lot variability from multiple sources. The outlier vials (vial 2 and 4) on PCs 1-3 had a calculated distance of 10.1 multidimensional standard deviations (SDs) (vial 2) from the center of the 11 other vials in lot AFN102, and 9.1 SDs (vial 4) from the center of the other 11 vials in the same lot (see Figure 3). Using a group membership range between 0 and 3 SDs, a standard deviation of 9 or 10 signals a potential quality control issue in lot AFN102.

Figure 4 is a close-up view of lot AFN102 in the region of 4100 to 4800 cm^{-1}. The orange spectrum that is labeled with wavenumbers is the spectrum of vial 2. This figure shows that the two peaks at 4524 and 4690 cm^{-1} are usually about the same size in the vials in lot AFN102.

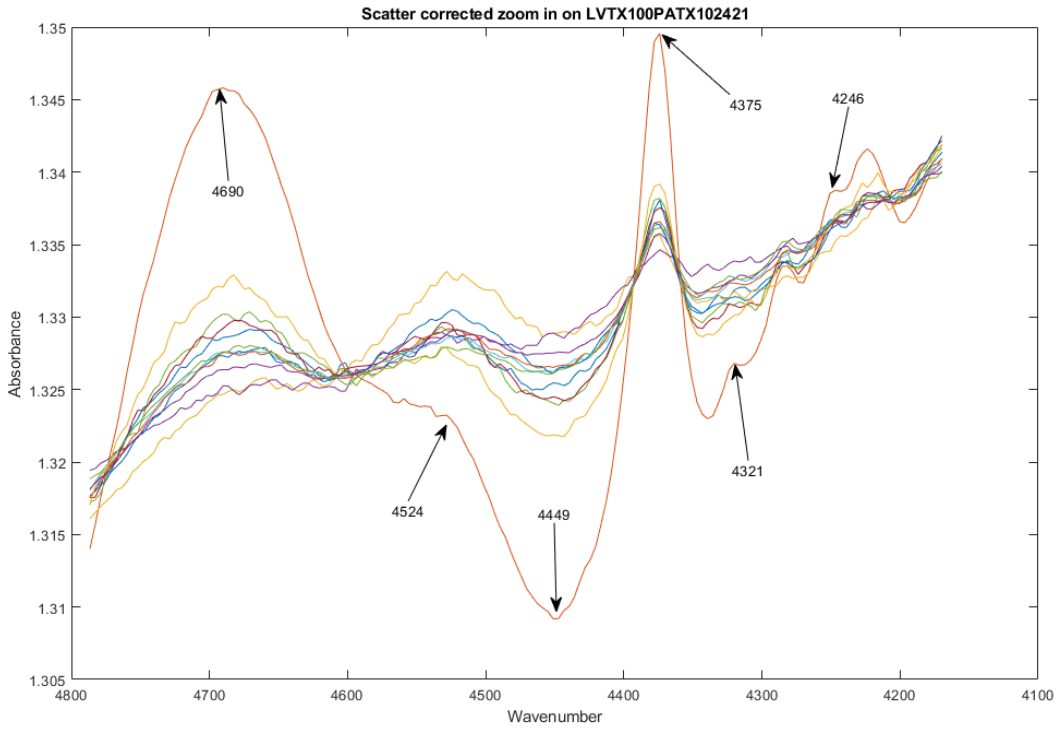

Figure 4. Zoom in on the spectra in Figure 1 in the region from 4100-4800 cm^{-1}. The spectra were scatter-corrected across this region for the figure. The orange line that is labeled with wavenumbers is vial 2.

The reduced noise level in vial 2 in Figure 4 is probably the result of a higher concentration of drug in the near-IR light beam in that vial.

Inter-Lot Analysis

Variations in the lots in the entire spectral library that are similar to those observed in lot AFN102 were also observed. There was substantial scatter in the spectra of each lot in the library (in part due to noise). However, there was also variability in the lots AFN001, AFN101, AFN103, AFN903, and AFN904 that was similar in nature to the variations observed inside lot AFN102 (see Figures 5, 6, and 7), albeit at lower frequency of occurrence.

The spectra of the PC loadings reveals how much each wavenumber contributes to the principal component score on that PC axis. For this reason, examining the loadings spectra of the PCs

can provide insight into the sources of variation and inhomogeneity in the different lots of material.

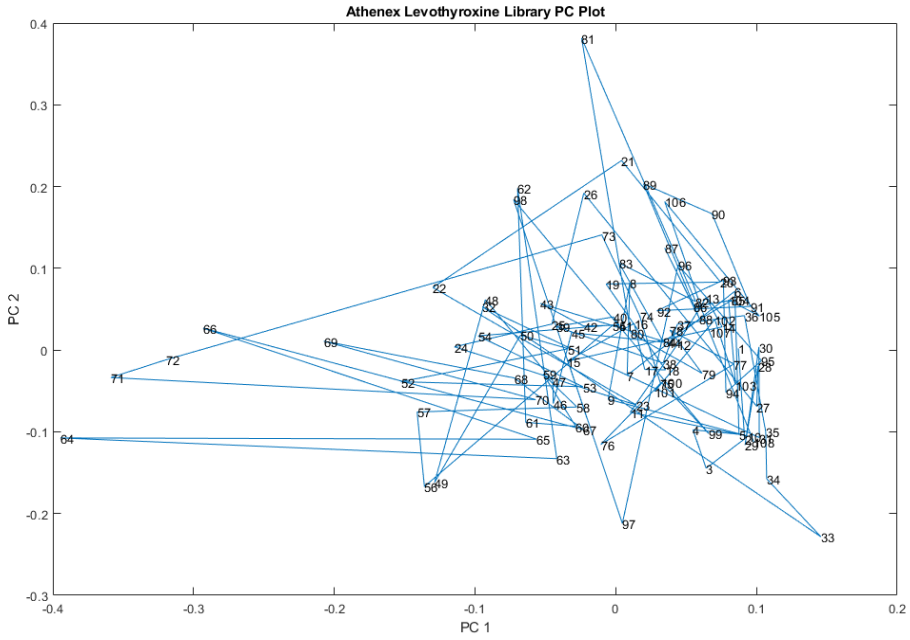

Figure 5. PC plot of the spectra in the library of Athenex levothyroxine vials. This spectral library comprises 108 samples from lots AFN001, AFN101, AFN102, AFN103, AFN903, and AFN904.

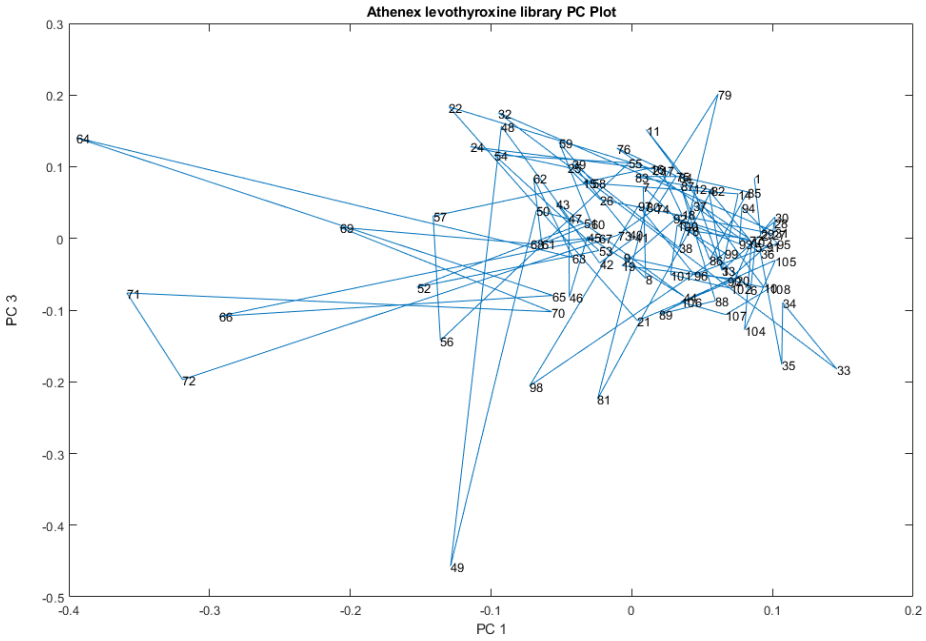

Figure 6. An orthogonal view of the PC scores of the spectra in Figure 5. Vials 49, 64, 66, 71, and 72 appear more like outliers in this view.

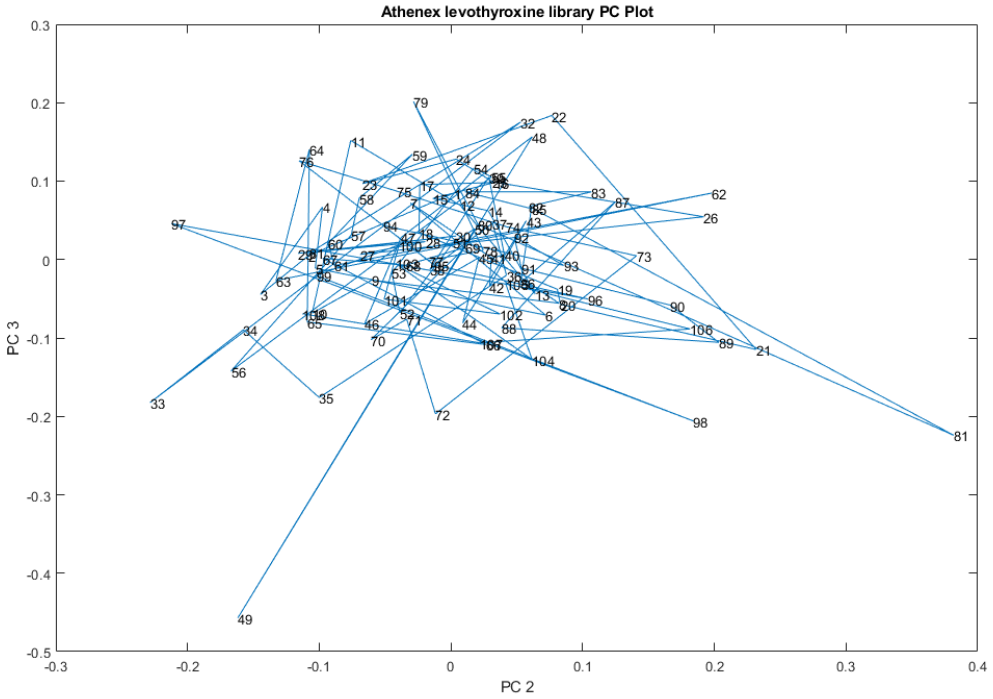

Figure 7. Another orthogonal view of the PC scores of the spectra in Figure 5 and Figure 6. In this view, spectra of vials 49 and 81 appear more like outliers.

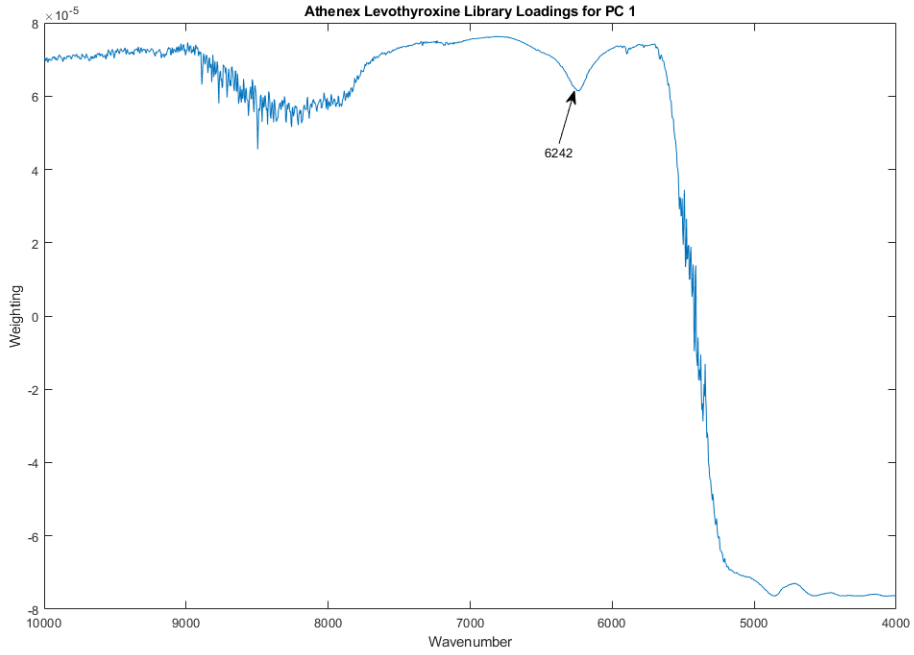

Figure 8. PC 1 loadings spectrum for the 108 levothyroxine vials in the library. See peaks in Figure 1.

The loadings of PC 1 in Figure 8 reveal mostly baseline variation, with only one strong spectral feature at 6242 cm^{-1}, and moderate noise.

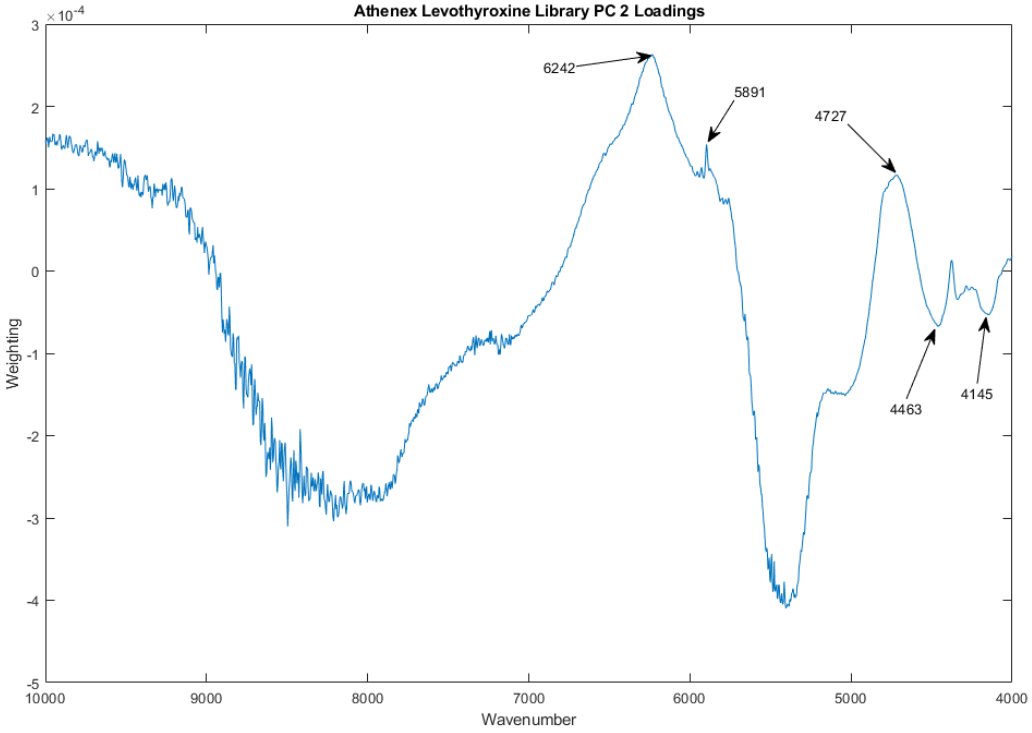

Figure 9. PC 2 loadings spectrum for the 108 levothyroxine vials in the library. See peaks in Figure 1.

In Figure 9 it is evident that the noise has increased in the loadings of PC 2, and baseline variation has decreased. But in contrast to PC 1 in Figure 8, there are now more spectral features. PC 2 contains important spectral features at 4145, 4463, 4727, 5505, 5891, and 6242 cm^{-1}.

In Figure 10, noise has spread across almost the entire loadings spectrum for PC 3. Nevertheless, spectral features are still observable at 4693, 5505, and 6242 cm^{-1}.

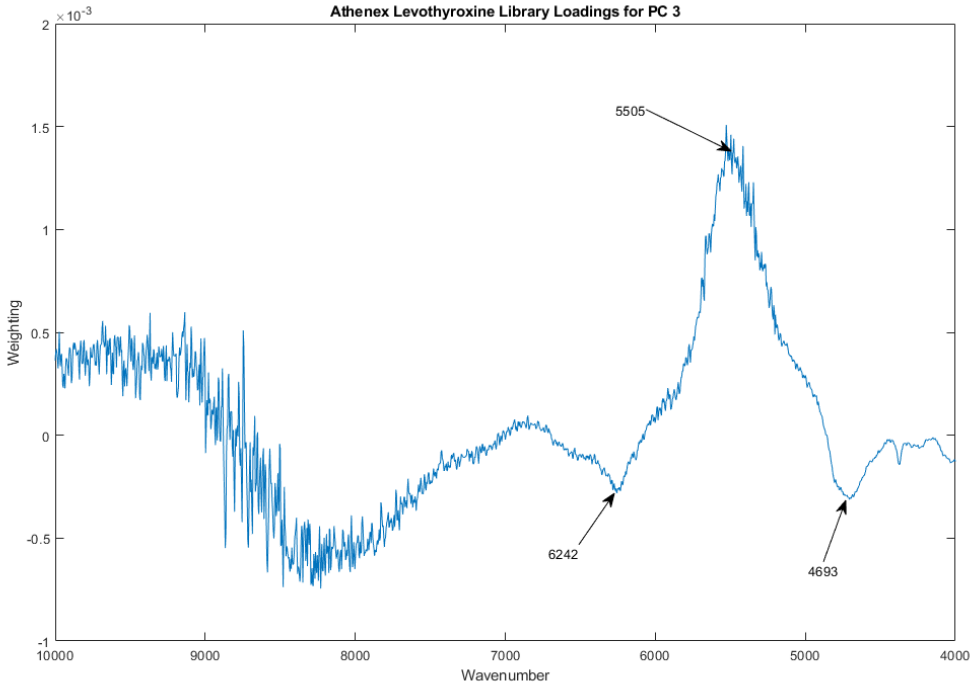

Figure 10. PC 3 loadings spectrum for the 108 levothyroxine vials in the library. See peaks in Figure 1.

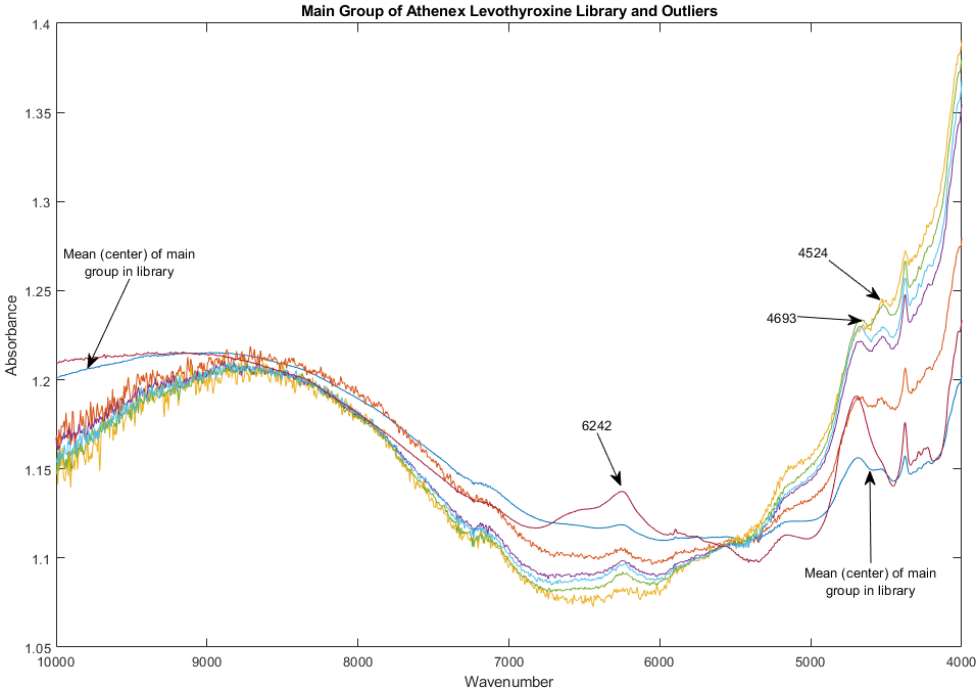

Figure 11. The mean spectrum of the main group in the library (i.e., center) is shown as a blue spectrum and marked at each end. In the main group of spectra, the peak at 4693 cm^{-1} is bigger than the peak at

4524 cm^{-1}. However, in most of the spectra in Lot AFN102 the two peaks are the same size, or the peak at 4524 cm^{-1} is larger. And there is one vial where the peak at 4693 cm^{-1} is so large it obscures the peak at 4524 cm^{-1}. Content uniformity appears to be an issue with the lots in the library.

Figure 11 is graphed in absorbance units instead of weightings like the previous three figures. In Figure 11 the mean spectrum of the main group in the library (i.e., the center point) is shown as a blue spectrum and annotated at each end. The outlier group spectra in Figure 11 (vials 49, 64, 66, 71, 72, and 81) are each plotted individually around the mean spectrum of the main group in the library. Figure 11 shows that there is major variability in the peaks at 4524 and 4693 cm^{-1}. The main library group mean spectrum shows that the peak at 4693 cm^{-1} is usually larger than the peak at 4524 cm^{-1}. However, in the outliers, that situation is more often reversed or does not exist. In 3 of the outlier spectra, the peaks at 4524 and 4693 cm^{-1} are the same size, while in 2 the peak at 4524 cm^{-1} is larger than the peak at 4693 cm^{-1}. In one spectrum, the peak at 4693 cm^{-1} is so large it almost completely covers the peak at 4524 cm^{-1} (see red line in Figure 11).

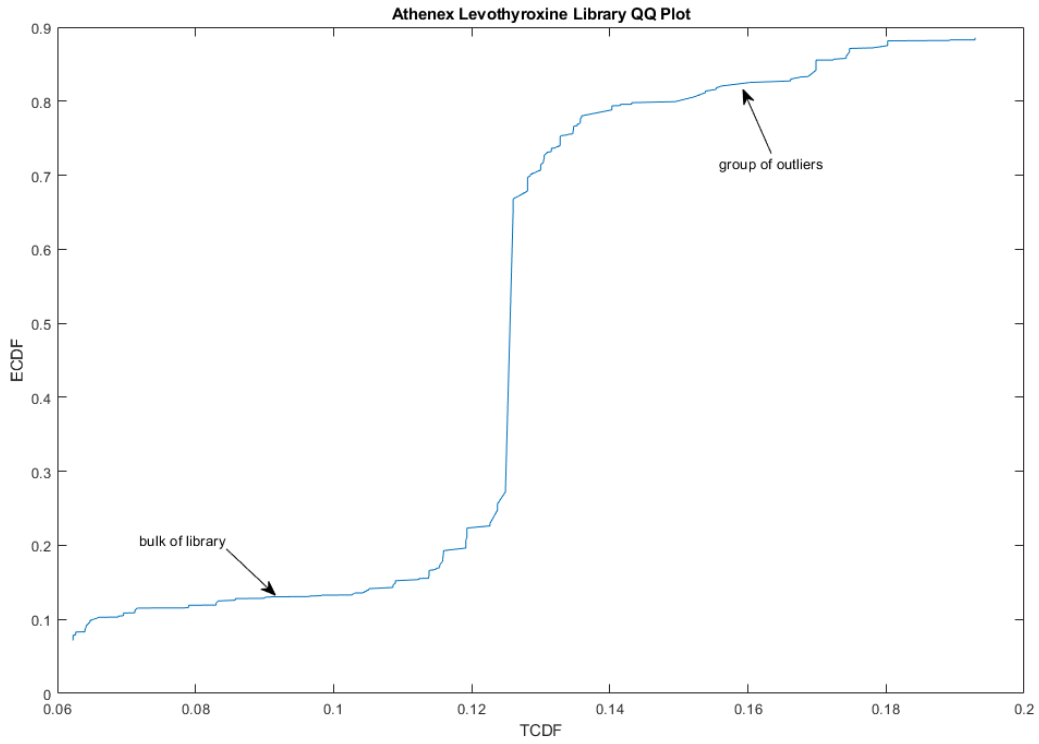

Figure 12. QQ plot of the main group and outliers for the levothyroxine library. The set of outliers have a different chemical composition (r_{lim}=0.99, r_t=0.88, p=0.02)

The QQ plot in Figure 12 shows a statistically significant difference in composition between the main group in the library and the outlier group (r_{lim}=0.99, r_t=0.88, p=0.02). The line segment for the group of outliers (upper part of graph) has a slightly greater slope than the line segment for the bulk of the library (lower part of graph), indicating that these 6 outliers are scatter across a

slight larger volume of hyperspace than the 102 spectra in the main library, and thus have greater variability than the main group in the library.

Conclusions

Statistically significant differences appear between vials in the same lot (intra-lot variation), and between different lots of the same product (intra-lot variation), for Athenex levothyroxine 100 µg. Two vials of 12 vials sampled from Athenex Lot AFN102 appeared 10.1 and 9.1 SDs from the center of the rest of the vials on the DQS FTNIR screening assay. Spectra of 108 vials from six lots in the library clustered in two groups (p=0.02), a larger main group of 102 vials and a smaller group of 6 outliers, suggesting they represent different material. Additional destructive testing should be conducted to determine whether these lots represent acceptable drug products.

Acknowledgements

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