Spectral Intra-Lot and Inter-Lot Variability in Carfilzomib

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RAPID COMMUNICATION

Abstract

Carfilzomib is a prescription injectable drug approved for use by the FDA as an antineoplastic agent, part of a drug class of medications known as proteasome inhibitors, and used to stop and slow the growth and progression of cancer cells within the body. The drug is approved as an agent to treat multiple myeloma. It is provided as a single-use vial that contains 60 mg of carfilzomib as a sterile, white to off-white lyophilized cake or powder.

Intra-lot and inter-lot variability in the spectra of carfilzomib vials was detected in the Drug Quality Study (DQS) using Fourier transform near-infrared spectrometry (FTNIR). One of 12 vials of lot 1143966 manufactured for Onyx Pharmaceuticals, Inc. appeared 4.7 multidimensional standard deviations (SDs) from the other 11 vials in a 3-D space formed by the first 3 principal components, which captured 81% of the total spectral variation.

Spectra of 168 vials from 18 lots in the spectral library formed two groups in the 3-D space formed by the first 3 principal components. One group contained 155 vials and the other group contained 13 vials. The 2 groups had different locations and scales using a subcluster detection test at $p=0.02$.

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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 (Westerfield, 2020) (Almeter, 2022). DQS currently screens medications, using FTNIR and Raman spectroscopy, for potential quality defects indicated by variability in absorbance peak intensities and locations. Through years of continuous monitoring, DQS has assembled a spectral library containing medications typically used in a health system 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 to FDA in an effort to hold manufacturers accountable for GMP requirements and to improve patient outcomes by exerting positive pressure on the pharmaceutical supply chain (Isaacs, 2022a). 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 (Isaacs, 2022d).

Carfilzomib is a selective proteasome inhibitor with a unique tetrapeptide epoxyketone structure (Arastu-Kapur, 2011). The major use case is as an agent to treat multiple myeloma. Generally, carfilzomib is administered alone or in conjunction with other drugs for the treatment of newly diagnosed, refractory, or relapsed multiple myeloma. A combination study found that carfilzomib, when administered with lenalidomide and dexamethasone, was efficacious in treatment of multiple myeloma (Franken, 2016).

A recent retrospective article analyzing clinical data of newly diagnosed multiple myeloma found carfilzomib used alongside other immunomodulator drugs may have a favorable safety profile (Landgren et al., 2019). However, administration of carfilzomib has also been noted to increase cardiovascular adverse events (CVAEs), some of which can be fatal. A meta-analysis evaluating carfilzomib toxicity data found administration of the drug was significantly associated with various CVAEs such as: ischemia, heart failure, hypertension, and arrhythmia (Waxman, 2017). Carfilzomib appears to be more effective than bortezomib, a first generation proteasome inhibitor, as far as treatment and toxicology profiles (Franken, 2016). The role of carfilzomib as a proteasome inhibitor is to irreversibly bind to proteasomes, preventing their function, and ultimately inducing apoptosis of myeloma cells. The accumulation of proteins due to proteasome inhibition has been shown to impede cell growth and cell division, disrupting cell function and leading to cell death (*New drug: Carfilzomib for multiple myeloma*, 2018).

Outcomes for patients with multiple myeloma have been improved in part due to the development of more effective proteasome inhibitors such as carfilzomib, as patients who are diagnosed within a five-year period have a survival rate of 55% (*Multiple myeloma - statistics*). Carfilzomib acts as both a selective and irreversible binder to the beta ring of the chymotrypsin-like 20S subunit of the proteasome (Groen, 2019). Currently, the literature does not provide any explanation for the cardiac adverse events that are associated with the injection of carfilzomib for treatment. Carfilzomib's appears able to cause the accumulation of misfolded protein, possibly in cardiomyocytes and that could potentially lead to adverse events. High doses may also adversely affect cardiac endothelial cells or increase toxicity (Groen, 2019). Screening methods for patients that are more likely to be at risk for adverse cardiovascular events are currently inadequate. As a result, carfilzomib use in patients requires monitoring.

Drug Product

Carfilzomib (Kyprolis, Amgen)(see Figure 1) is approved for use by the FDA as an antineoplastic agent. Structurally the drug is a tetrapeptide epoxyketone (Arastu-Kapur, 2011). The safety of this drug in children under the age of 18 is unknown. Cardiovascular adverse events associated with carfilzomib indicate that elderly patients as well as those with cardiovascular issues on the drug should be monitored.

Figure 1. Vials of Kyprolis®(carfilzomib) for injection from lot 1143966. The drug appears as a white to off- white freeze-dried powder or cake. The vials were scanned nondestructively in the near-infrared spectral region through the bottom of the vials.

The lots comprising the spectral library for carfilzomib were 1111311, 1111312, 1114656, 1115367, 1122726, 1125073, 1129112, 1130633, 1133559, 1133560, 1133627, 1133641, 1134256, 1135205, 1136598, 1136600, 1138123, and 1138124.

Related Reports

In 2016 Amgen initiated a voluntary recall of Kyprolis® 30 mg vials (carfilzomib) due to possible cracks in the glass of the vials (https://ndclist.com/ndc/76075-103/recalls)

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected for inventory belonging to lot 1143966 as part of routine medication quality screening. A representative sample of 12 individual vials were selected for screening from lot 1143966 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).

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

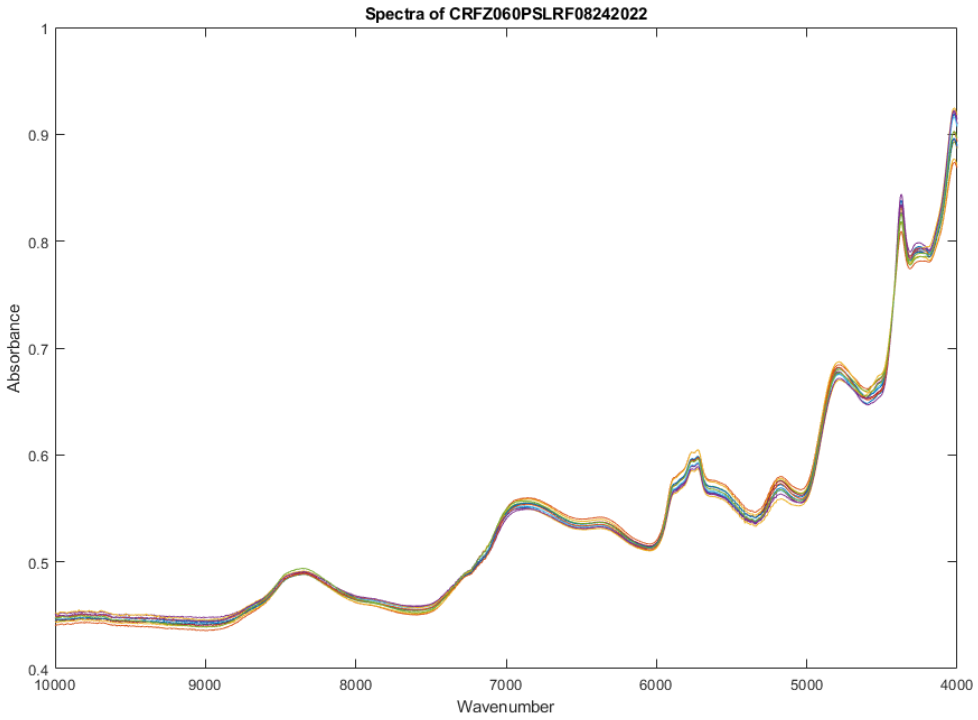

Figure 2. FTNIR spectra of 12 vials from lot 1143966 of carfilzomib.

Spectra from 12 vials from lot 1143966 of carfilzomib appear in Figure 2. The spectra appear superficially similar but there are slight differences in the peaks between 4100 cm^{-1} and 4900 cm^{-1}. These differences were enough to appear on a principal component lot of PCs 1 through 3 (see Figure 3). PCs 1-3 together account for 81% of the total spectral variation (see Table 1). Vials 10, 11, and 12 appear separated from the rest, but only vial 10 was separated out by the BEST algorithm, falling 4.7 multidimensional standard deviations (SDs) away from the other 11 vials in the space defined by the first 3 PCs.

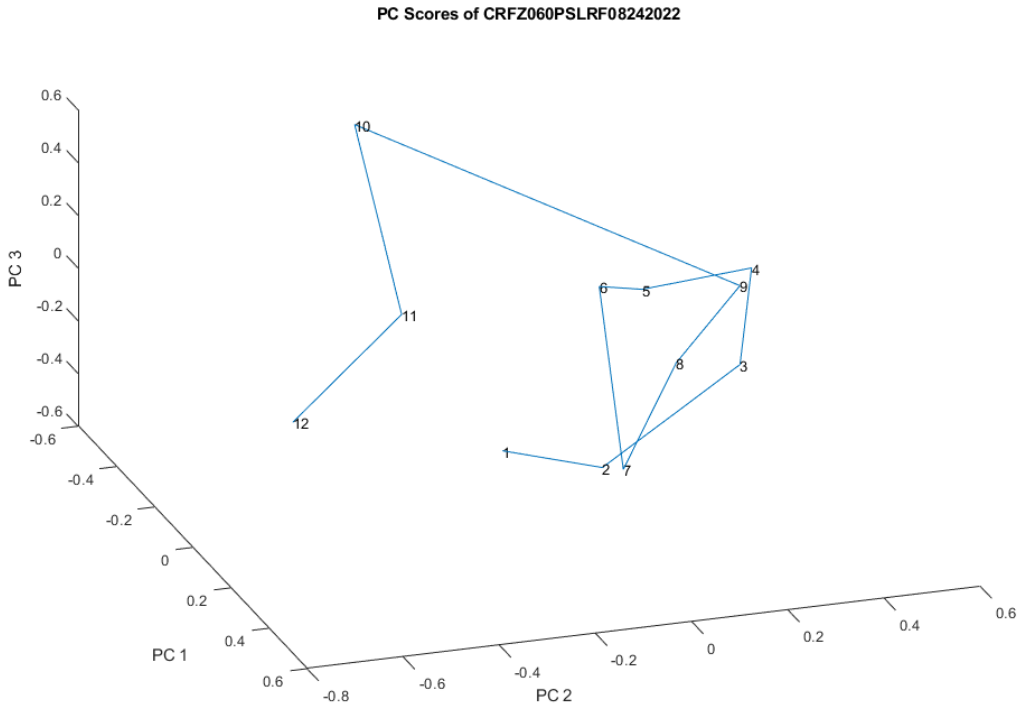

Figure 3. PC plot of the spectra of Lot 1143966 of carfilzomib shown in Figure 2. Vial 10 is located 4.7 BEST SDs from the other 11 vials.

Table 1. Variation accounted for by each of the PCs of the spectra of Lot 1143966

PC Number	Variation in this PC	Cumulative PC Variation
1	0.4956	0.4956
2	0.2003	0.6959
3	0.1100	0.8059
4	0.0908	0.8968
5	0.0255	0.9223
6	0.0226	0.9448

Interlot Analysis

Spectra of the 167 vials in the carfilzomib library are shown in Figure 4. Figure 4 is a zoom-in on the region of interest from Figure 2. Small differences in peaks are noted at 4254 and 4527 cm^{-1}.

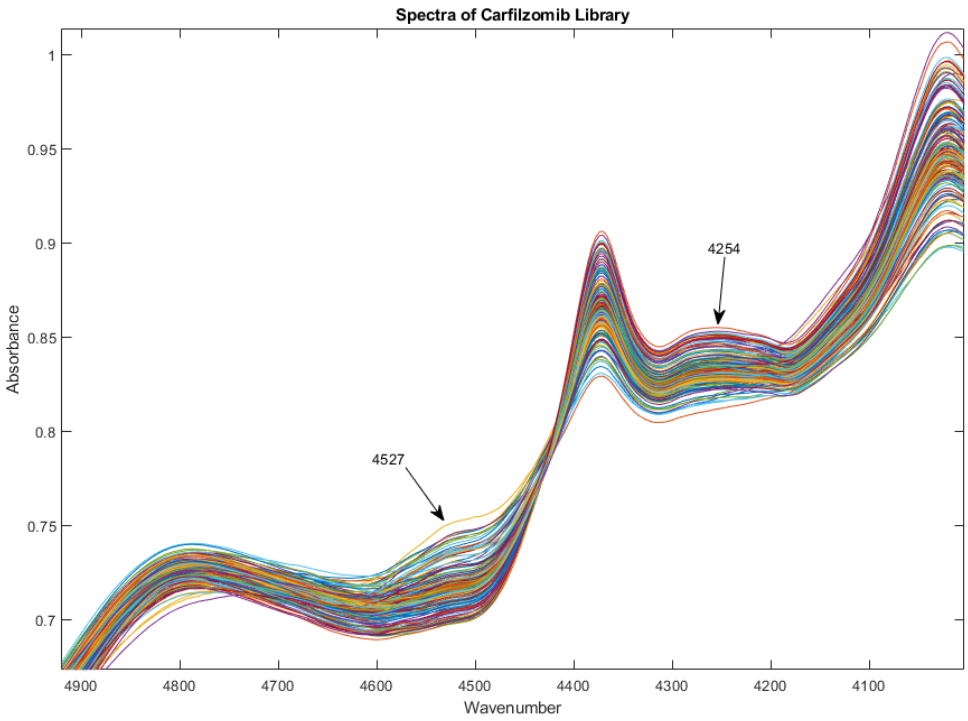

Figure 4. Spectra of carfilzomib library including a total number of 167 vials. Peak differences are marked at 4254 and 4527 cm^{-1}, and a peak shift at 4800 cm^{-1} also appears in some spectra.

Figure 5 is a PC plot of the spectra of the 167 vials from 18 lots showing the spectra lie in 2 adjacent distinctive regions in hyperspace. PCs 1-3 are plotted, and these PCs together account for 67% of the total spectral variation. The smaller group contains 13 vials (7.8% of the total number of vials), while the larger group contains 154 vials.

Figure 6 is a rotation of Figure 5 that shows a few other vials (e.g., vials 149 and 156) also tend to fall outside the main cluster.

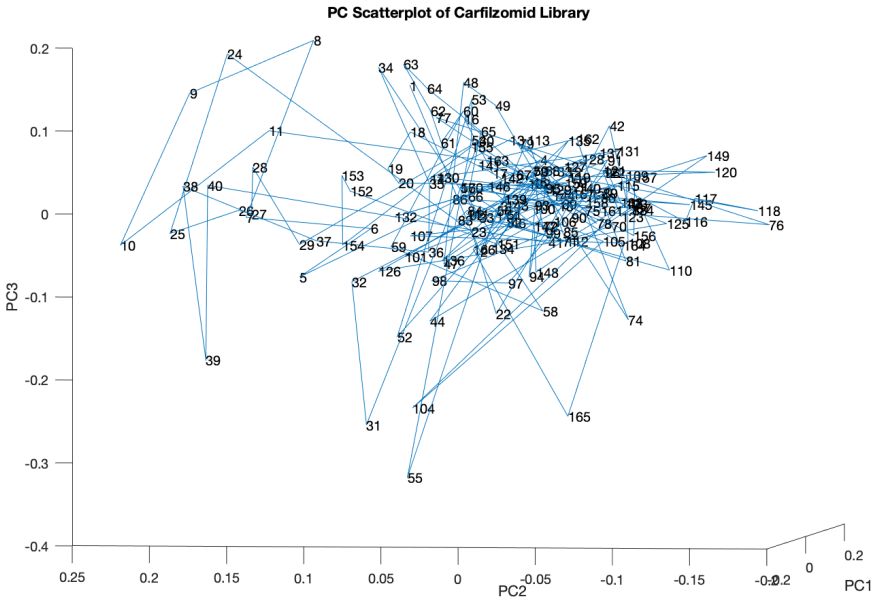

Figure 5. Spectra of the 167 vials in the spectral library from 18 lots with 2 distinctive regions in hyperspace. The thirteen outlier vials in the left-most region (7, 8, 9, 10, 11, 24, 25, 26, 27, 28, 38, 39, 40) are noted as being a part of a distinct region of hyperspace (7.8% of the total number of vials).

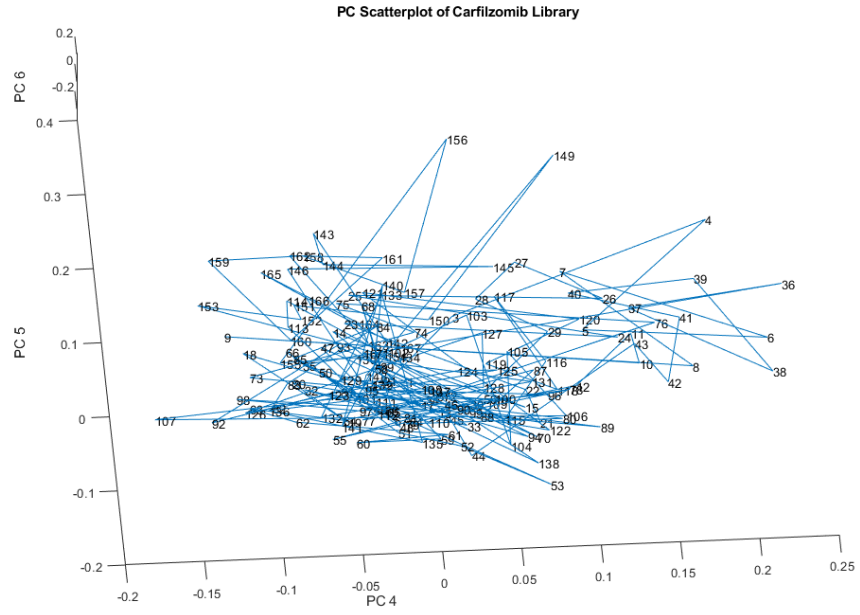

Figure 6. Spectra of the 167 vials in the spectral library shown in Figure 5, but with a different rotation. This figure shows a few other vials (e.g., 149 and 156) also tend to fall outside the main cluster.

Table 2. Variation accounted for by each of the principal components of the spectra of the carfilzomib library.

PC Number	Variation in this PC	Cumulative PC Variation
1	0.4122	0.4122
2	0.1647	0.5769
3	0.0971	0.6740
4	0.0748	0.7488
5	0.0289	0.7776
6	0.0221	0.7997

Table 2 shows the variation accounted for by each of the principal components of the spectra of the carfilzomib Library. The first three PCS now account for about 67% of the spectral variation, down from 81% in just Lot 1143966.

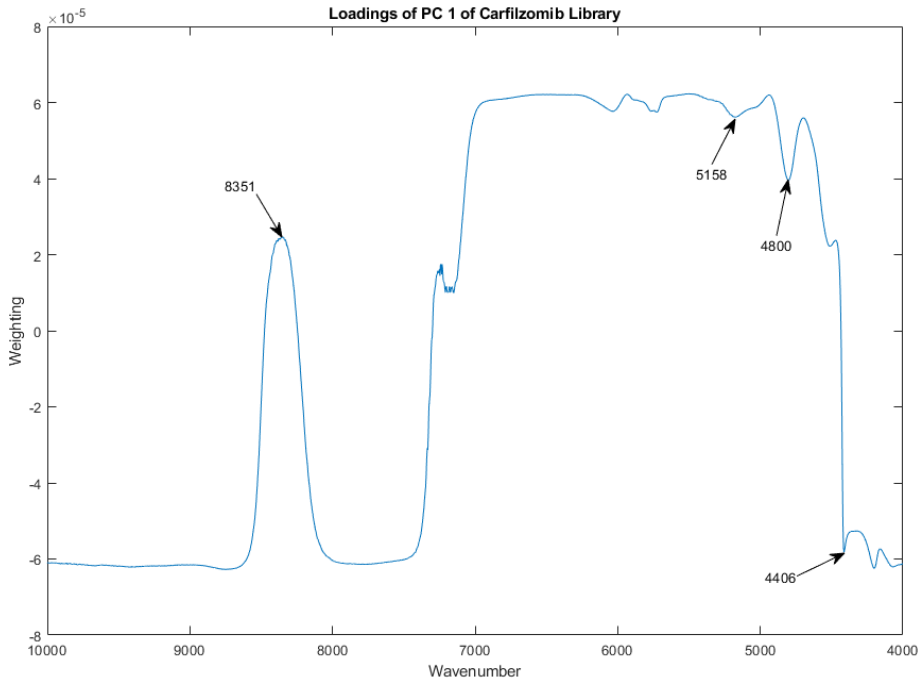

Figure 7. Plot of the PC loadings spectrum for PC 1 of the spectral library for carfilzomib. As is often the case, the first PC captures mostly baseline variations following multiplicative scatter correction.

Figure 7 shows a plot of the principal component loadings spectrum for PC1 of the spectral library for carfilzomib. Peak changes at 4800, 5158, and 8351 cm^{-1} are inversely related to peak changes at 4406 cm^{-1}.

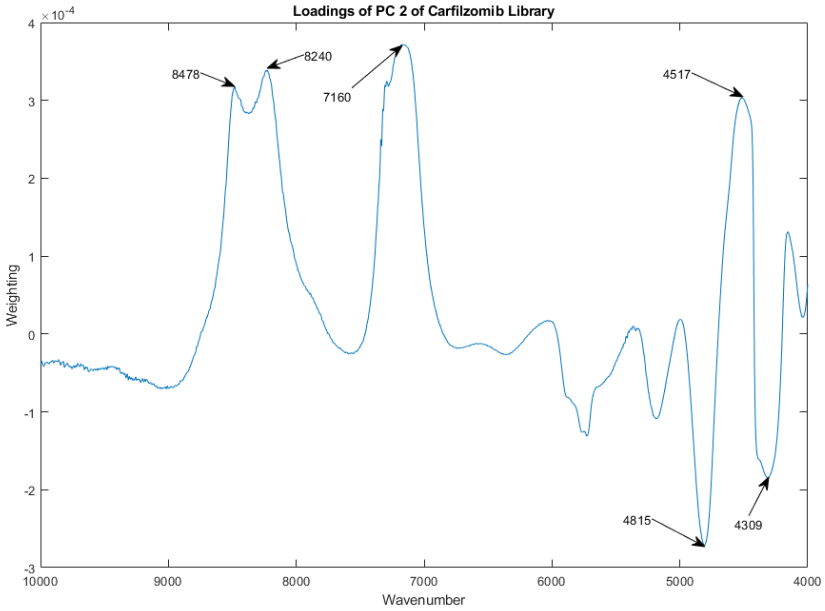

Figure 8. Plot of the loadings spectrum for PC 2 of the spectral library for carfilzomib.

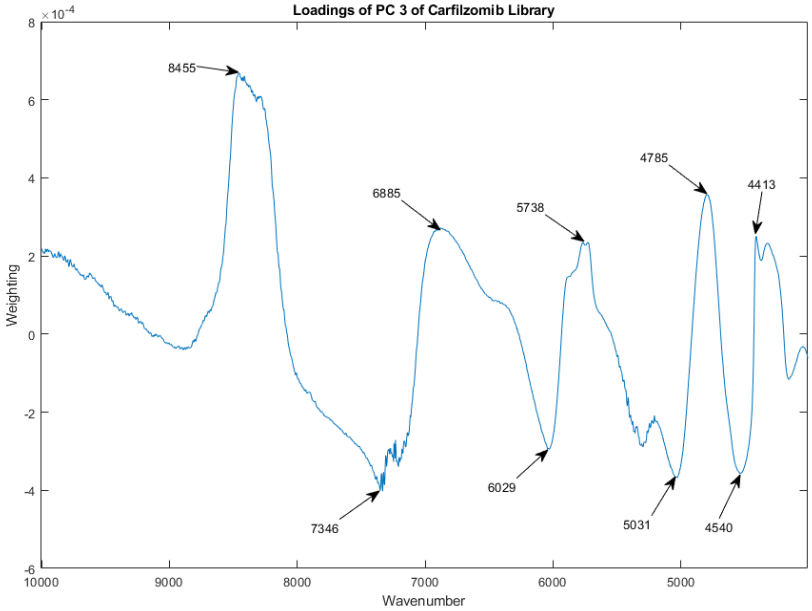

Figure 9. Plot of the loadings spectrum for PC 3 of the spectral library for carfilzomib.

Figure 8 is a plot of the loadings spectrum for PC2 of the spectral library for carfilzomib. Peak changes at 4517, 7160, 8240, and 8478 are inversely related to peak changes at 4309 and 4315 cm^{-1}. Figure 9 is a plot of the loadings spectrum for PC 3 of the spectral library for carfilzomib. Peak changes at 4413, 4785, 5738, 6885, and 8455 cm^{-1} are inversely related to peak changes at 4540, 5031, 6029, and 7346 cm^{-1}. Together, PCS 1, 2, and 3 account for 67.4% of the total spectral variation of the carfilzomib library.

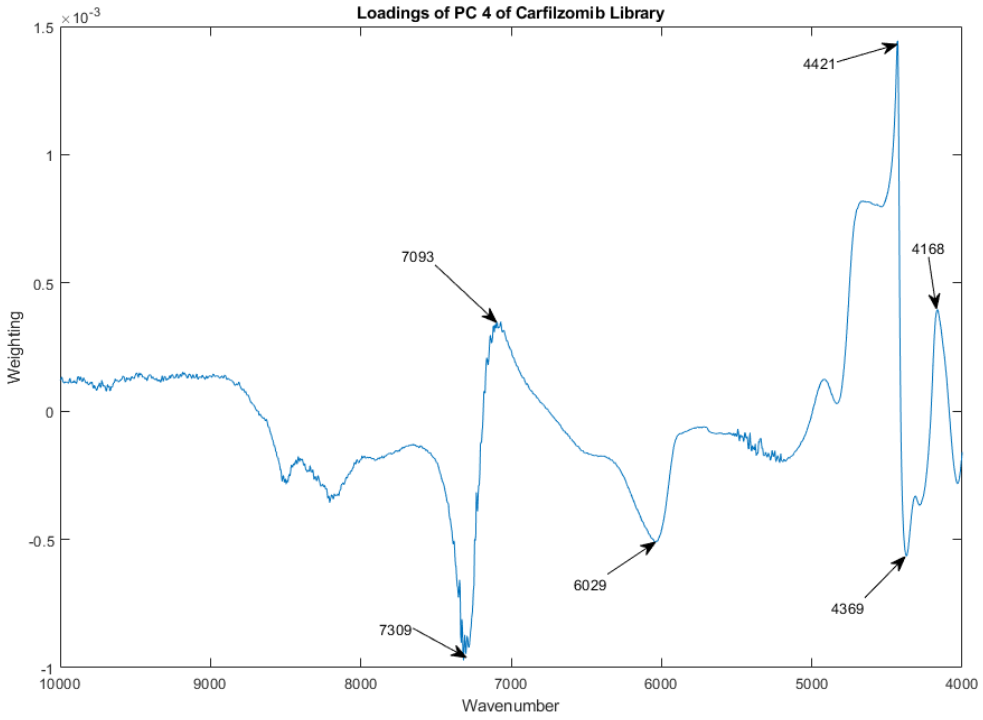

Figure 10. Plot of the loadings spectrum for PC 4 of the spectral library for carfilzomib.

Figure 10 is a plot of the loadings spectrum for PC4 of the spectral library for carfilzomib. Peak changes at 4168, 4421, and 7093 cm^{-1} are inversely related to peak changes at 4369, 6029, and 7309 cm^{-1}.

Figure 11 is a plot of the loadings spectrum for PC5 of the spectral library for carfilzomib. Peak changes at 4428, 4748, 5180, 7346, and 8485 cm^{-1} are inversely related to peak changes at 4540 and 6125 cm^{-1}. Figure 12 presents a plot of the loadings spectrum for PC6 of the spectral library for carfilzomib. Peak changes at 4421, 4733, and 7331 cm^{-1} are inversely related to peak changes at 5173 and 7063 cm^{-1}. Together, PCS 4, 5, and 6 describe 13.9% of the total spectral variation.

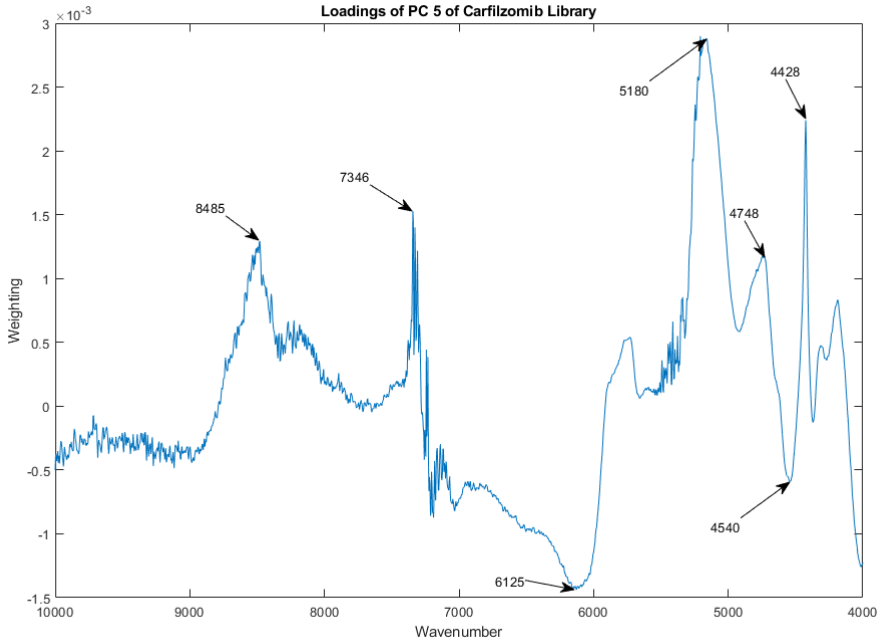

Figure 11. Plot of the loadings spectrum for PC 5 of the spectral library for carfilzomib. Noise is beginning to appear in the spectra from the high wavenumber end of the spectrum.

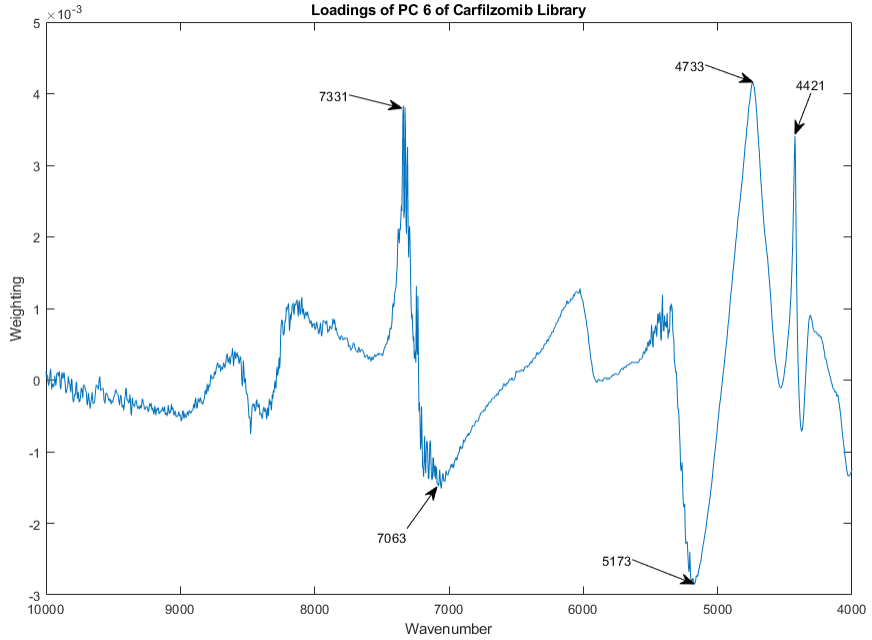

Figure 12. Plot of the loadings spectrum for PC 6 of the spectral library for carfilzomib.

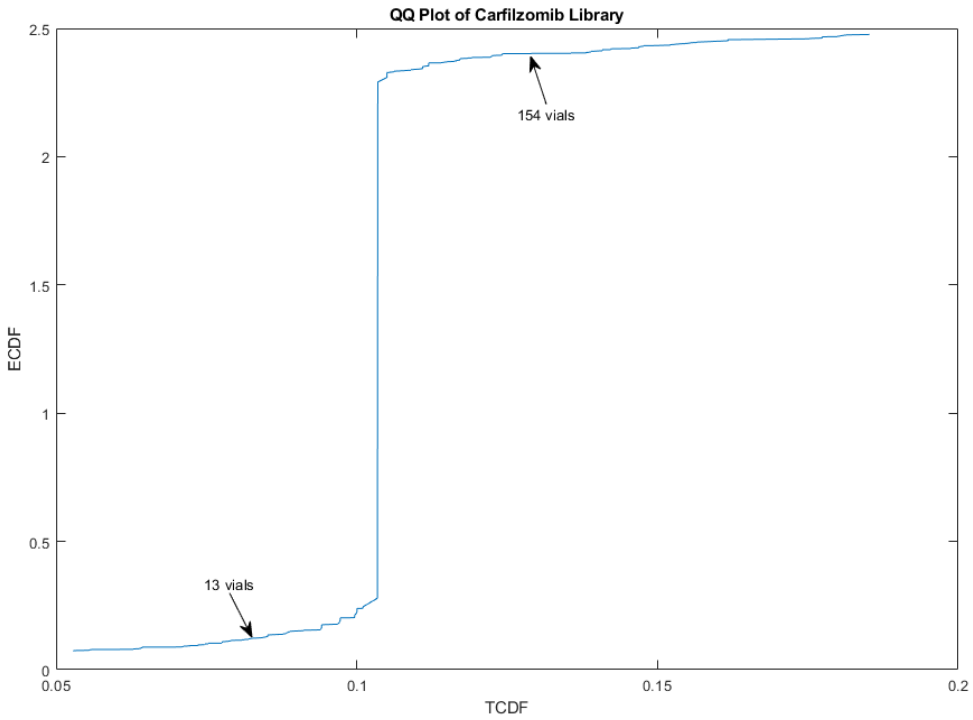

Figure 13. The QQ plot of the carfilzomib library using the subcluster detection method (r_{lim}=0.98, r_{tst}=0.84, p<0.02). The test was performed in the three-dimensional space formed by PCS 1, 2, and 3.

Figure 13 shows the QQ plot from the subcluster detection test conducted on the carfilzomib library of 167 vials in a three-dimensional space using the first three PCs. There are 13 vials in one group and 154 vials in the other group. The two groups are statistically different (r_{lim}=0.98, r_{tst}=0.85, p<0.02). The mean correlation coefficient for the bootstrap samples of the training set was 0.99 with SD=0.004, making the 13 vials 36.3 SDs of the correlation coefficient away from the mean value of the correlation coefficient for the 154 vials.

Conclusions

Carfilzomib is a prescription injectable drug approved for use by the FDA as an antineoplastic agent, part of a drug class of medications known as proteasome inhibitors, and used to stop and slow the growth and progression of cancer cells within the body. The drug is approved as an agent to treat multiple myeloma. There was a previous report of a quality problem involving cracked vials and carfilzomib.

Intra-lot and inter-lot variability in the spectra of carfilzomib vials was detected in the Drug Quality Study (DQS) using Fourier transform near-infrared spectrometry (FTNIR). One of 12 vials of lot 1143966 manufactured for Onyx Pharmaceuticals, Inc. appeared 4.7

multidimensional standard deviations (SDs) from the other 11 vials in a 3-D space formed by the first 3 principal components, which captured 81% of the total spectral variation. Spectra of 168 vials from 18 lots in the spectral library formed two groups in the 3-D space formed by the first 3 principal components. One group contained 155 vials and the other group contained 13 vials. The 2 groups had different locations and scales using a subcluster detection test at p=0.02.

Quality control is important in drug manufacturing. Good drugs lead to good patient outcomes. These FTNIR 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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