Contact in Context ***CIC Pharmaceutical Sciences***

Spectrometric Analysis of Dantrolene Sodium

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RAPID COMMUNICATION

Abstract

Dantrolene sodium is a direct-acting skeletal muscle relaxant. Dantrolene sodium for injection is indicated, along with suitable supportive measures, for the management of sudden, severe hypermetabolism of skeletal muscle typical of malignant hyperthermia crises in patients of any age. The formulation scanned in this work was designed to be injected intravenously.

Intra-lot and inter-lot variability in the spectra of REVONTO™ (dantrolene sodium) was measured in the Drug Quality Study (DQS) using Fourier transform near-infrared spectrometry (FTNIR). Spectra of 69 vials from lot 20REV01A contained two groups (n_1=56 vials, n_2=13 vials) when scanned with an FTNIR. The two groups of spectra in lot 20REV01A were found to be 66.7 SDs apart using a subcluster detection test, suggesting that the two groups were manufactured differently. As a result, all available samples of dantrolene were examined.

A library of spectra of 141 vials of dantrolene from 4 lots were found to contain 3 separate groups, also suggesting that different vials contain different materials.

Introduction

The University of Kentucky's (UK) Drug Quality Study was established in August of 2019 to engage in consumer-level quality assurance screening for drugs used within UK HealthCare's pharmacies. DQS 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, DQS has assembled a spectral library containing medications typically used in a health system setting. Statistical analyses using DQS' 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, DQS 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

Dantrolene sodium is a direct-acting skeletal muscle relaxant. Dantrolene sodium for injection is indicated, along with suitable supportive measures, for the management of sudden, severe hypermetabolism of skeletal muscle typical of malignant hyperthermia in patients. A picture showing the labeling of 2 vials of dantrolene sodium from lot 20REV01A appears in Figure 1. Vials from lots 20REV01A, 19REV07A, 19REV08A, and 19REV12A were used in this study.

Background

Current research is underway using dantrolene. For example, hyperthermia induced by psychomotor stimulants may cause leakage of the blood-brain barrier, vasogenic edema, and lethality in extreme cases. Current treatments such as whole-body cooling only address symptoms and a clear need to develop pharmacological interventions exists. Dantrolene sodium, a peripheral muscle relaxant used in the treatment of malignant hyperthermia, has been proposed as potentially effective to treat MDMA-hyperthermia in emergency rooms. (Cameron-Burr, 2023) However, debate around its efficacy for this indication persists. To investigate dantrolene as a treatment for hyperthermia induced by illicit psychomotor stimulant drugs, Ryanodex®, a concentrated formulation of dantrolene sodium produced by Eagle Pharmaceuticals, was tested with 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (METH)-induced hyperthermia in awake freely moving rats.

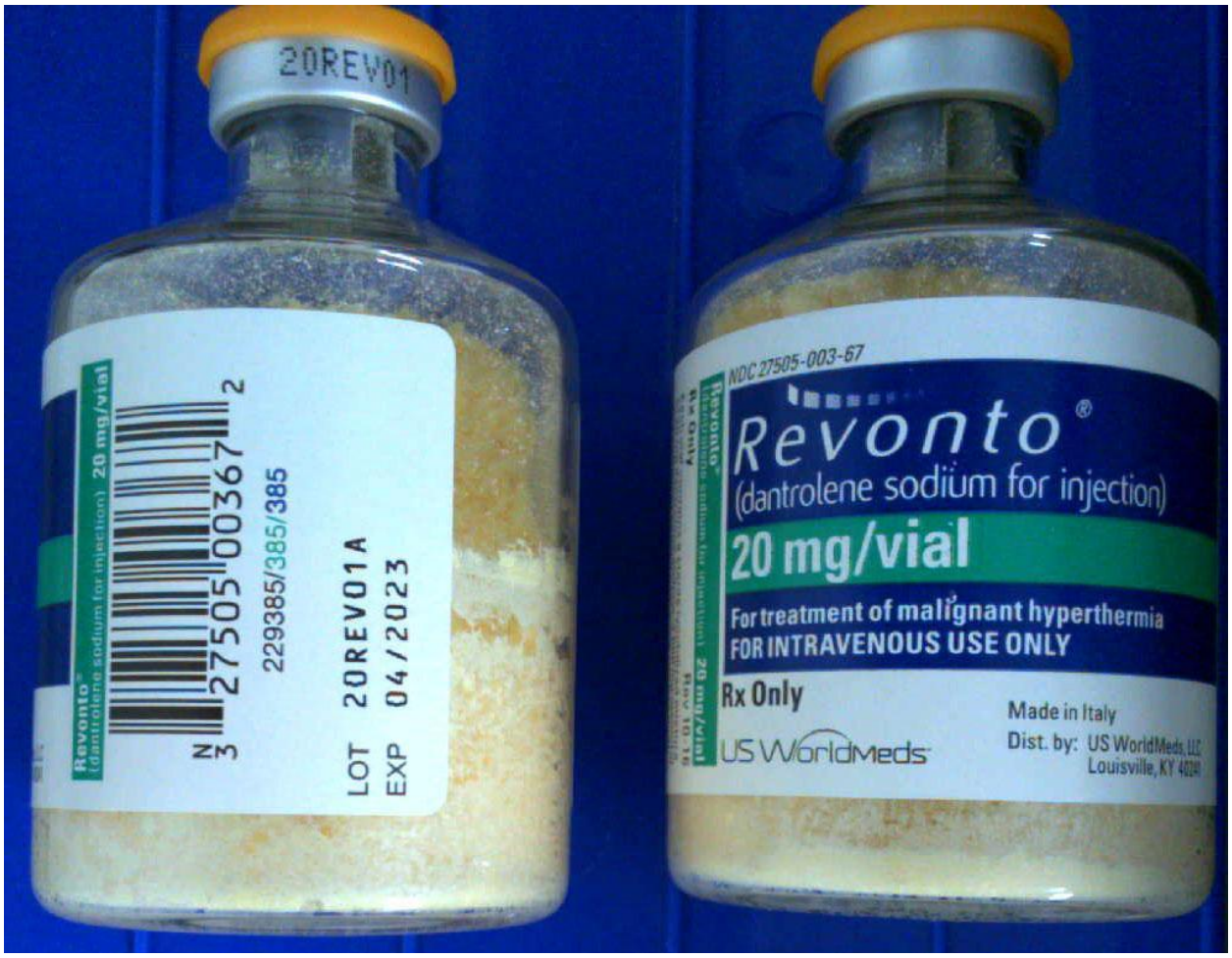

Figure 1. Vials of dantrolene sodium for injection. These vials are from lot 20REV01A.

Dantrolene failed to attenuate MDMA- and METH-induced hyperthermia, though locomotor activity was significantly reduced. All animals maintained at warm ambient temperatures that received dantrolene during severe drug-induced hyperthermia died within or soon after the recording session. The results suggest that dantrolene sodium formulations are not mechanistically suited to treat MDMA- and METH-induced hyperthermia.

Dantrolene is also a ryanodine receptor antagonist (Samiotis, 2022). The impairment of intracellular calcium homeostasis plays an essential role during ischemia-reperfusion injury. Calcium release from sarcoplasmic reticulum that is triggered by myocardial ischemia is mainly mediated by ryanodine receptors. An in vivo, experimental trial comparing 10 experimental swine that received dantrolene sodium with 9 control swine was conducted. Their left anterior descending coronary artery was temporarily occluded for 75 minutes via a vessel tourniquet, which was then released. Myocardial reperfusion was allowed for 24 hours. Dantrolene was

administered at the onset of the reperfusion period and levels of troponin, creatine phosphokinase and creatine kinase myocardial band between the two groups were compared. There were significantly lower values of troponin, creatine phosphokinase and creatine kinase myocardial band in the dantrolene group, indicating less ischemia-reperfusion injury. Moreover, the postischemic cardiac index was also greater in the dantrolene group, and viable myocardium was also better preserved. An in vivo cardioprotective role of dantrolene sodium against ischemia-reperfusion injury in swine models was indicated in this study. Eventually, dantrolene sodium administration could be a promising treatment against ischemia-reperfusion injury in humans. However, large randomized clinical studies should be carried out to prove this hypothesis.

In 2019 there was an active recall of Revonto (dantrolene sodium) from the lot 17REV01 vials. 6456 vials were recalled due to the appearance of the solution. This came to light during 24-month stability testing (Tumolo, 2019).

FDA Medwatch

An FDA Form 3500 Medwatch describing the findings of this Rapid Communication was filed on Jun. 7, 2023.

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected for inventory belonging to lots 19REV12A and lot 20REV01A as part of routine medication quality screening. The vials were 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, 2022b).

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).

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, 2023)(Pollock, 1998).

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 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 (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

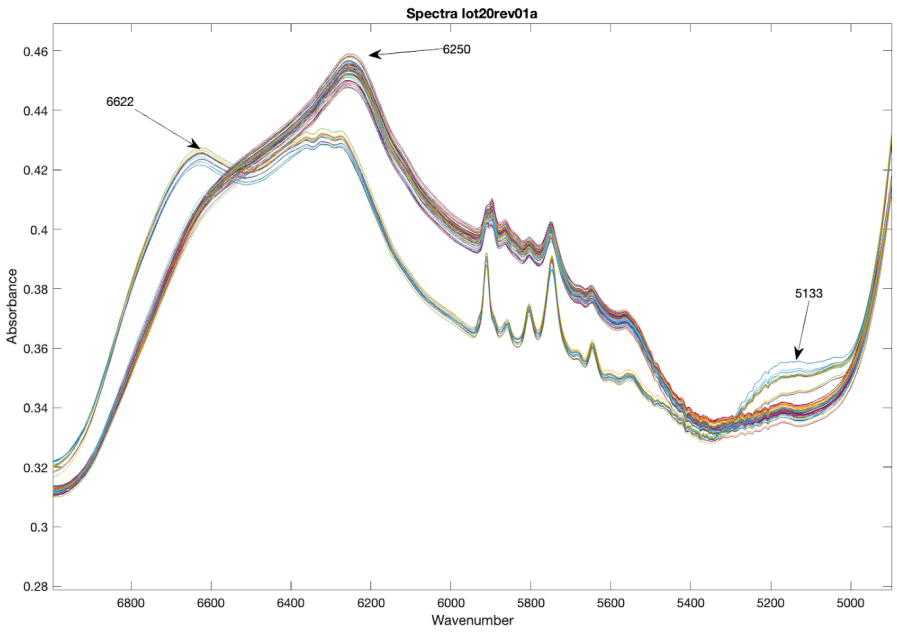

Figure 2.The figure shows Fourier transform near-infrared (FTNIR) spectra of 69 vials from lot 20REV01A in the range of 5000–6800 cm^{-1}. The labeled peaks are indicative of major differences within one lot of the drug. The variation in their intensity suggests that there is some variation in the drug's composition between the vials.

The spectra from one lot of dantrolene vials (lot 20REV01A) are presented in Figure 2. It appears that lot 20REV01A contains two distinct groups, which will be shown in the principal component scatterplots. Each group has its own set of peaks, and even when similar peak patterns are observed, the magnitude varies greatly between the groups.

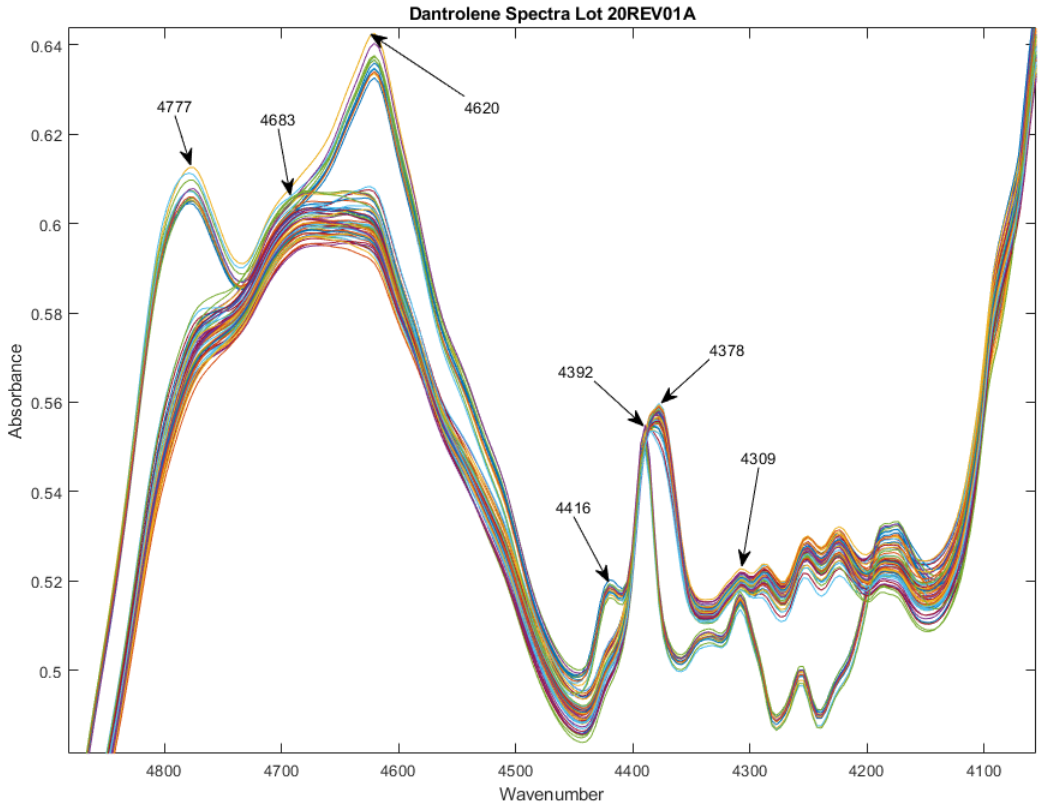

Figure 3. FTNIR spectra of 69 vials from lot 20REV01A from 5000-4100 cm^{-1}. The peaks with the most variation are labeled according to their wavenumber.

Figure 3 depicts another region of the spectra in which there are significant variations between the vials of dantrolene. As seen in Figures 2 and 3, there are two distinct groups, each with its own unique set of peaks. The peak differences shown in Figure 3 are just as dramatic as in Figure 2, particularly the peaks labeled 4777 and 4620 cm^{-1}. Important differences also exist in the region of 4200 to 4300 cm^{-1}.

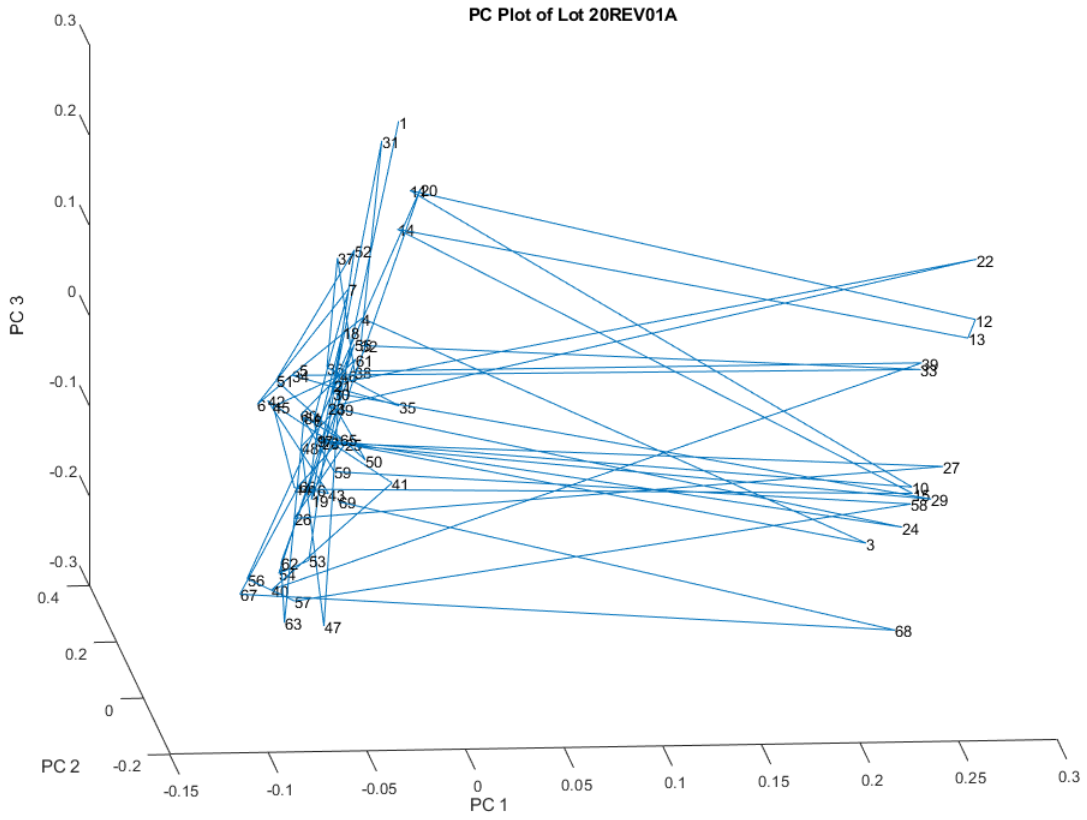

Figure 4. A principal component (PC) scatterplot of 69 vials from Lot 20REV01A shows two distinct groups. The blue line indicates that the outliers in the group on the right are dispersed throughout the 69 samples taken from the lot. These three PCs account for 62% of the total spectral variation.

Figure 4 is a principal component scatterplot that illustrates the separation of the two distinct groups in Lot 20REV01A. The subcluster detection test found that these groups are separated by 66.7 SDs. The QQ plot for the subcluster detection test is shown in Figure 5. The cluster on the right contains 13 vials, while the cluster on the left contains 56 vials. Approximately 19% of the vials are in the group on the right. Note that two vials that appear to be in the left group, vials 35 and 41, are slightly displaced in the direction of the group on the right.

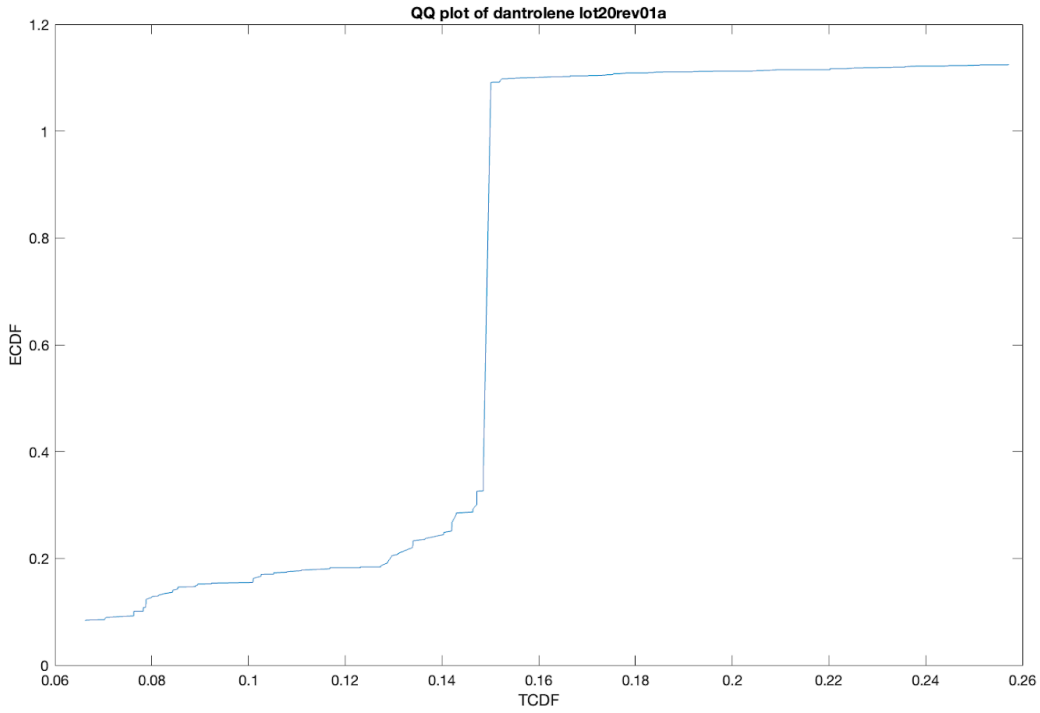

Figure 5. The QQ plot of Lot 20REV01A of dantrolene. The distance between the two groups highlights that the groups likely are not exactly the same material.

Table 1 shows the individual and cumulative variations accounted for by the six largest principal components of the spectra of lot 20REV01A. The principal components were calculated after multiplicative scatter correction.

Table 1. Variation accounted for by each of the 6 largest principal components of the spectra of lot 20REV01A.

PC Number	Variation in this PC	Cumulative PC Variation
1	0.3203	0.3203
2	0.1974	0.5177
3	0.1036	0.6213
4	0.0701	0.6914

5	0.0554	0.7469
6	0.0381	0.8047

Interlot Analysis

Seventy-two additional vial spectra were available in the spectral library from other lots of US Worldmeds dantrolene (lots 19REV07A, 19REV08A, and 19REV12A). These spectra were added to the spectra of lot 20REV01A, scatter corrected, and principal components were calculated. A PC scatterplot of the resulting dantrolene library appears in Figure 6.

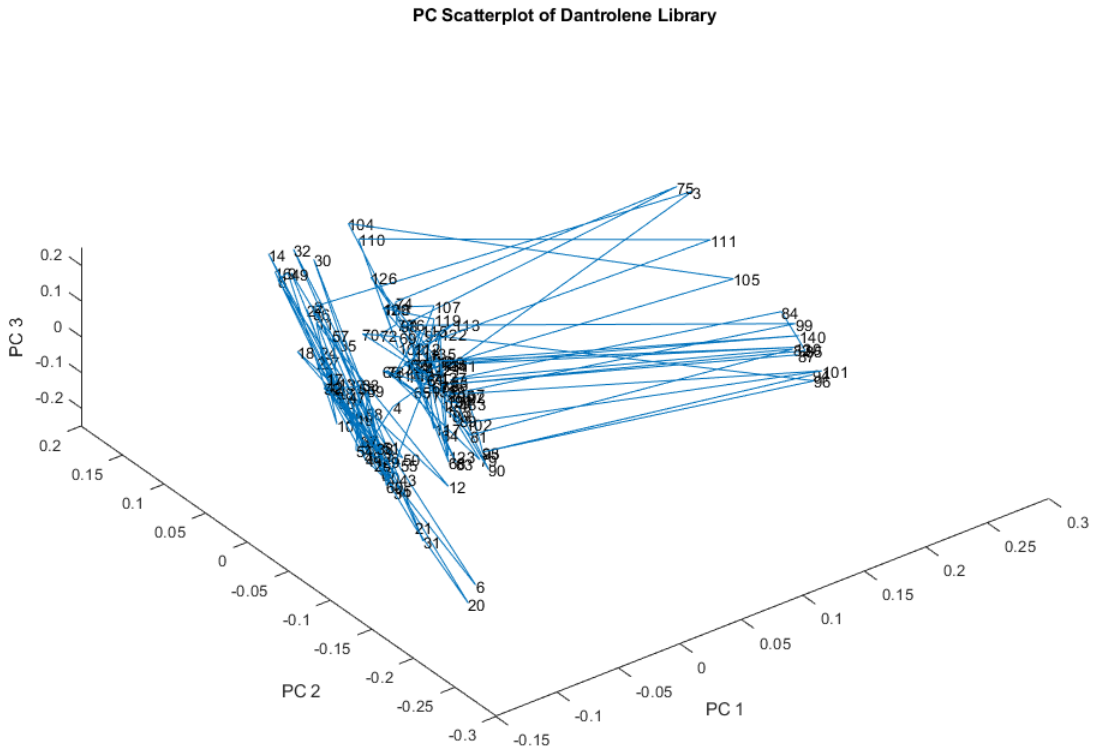

Figure 6. PC scatterplot of the 141 vials in the dantrolene library on PCs 1, 2, and 3. In this plot, three separate groups are apparent. These three groups might have also been present in Figure 4, but only represented by vials 35 and 41. Group 1 is the group farthest to the left, and is the blue line in Figure 8. Group 2 is just to the right of group 1, in between groups 1 and 3. Group 2 is the red line in Figure 8. Group 3 on the right forms the yellow line in Figure 8. These three PCs account for 61% of the total spectral variation.

Figure 6 shows three distinct groups of dantrolene formulations in the library. The groups can be identified as 1, 2, and 3, numbering from from left to right. Group 1 contains 50 vials, group 2 contains 78 vials, and group 3 contains 13 vials. The distance between groups 1 and 2 is 26.2 standard deviations (SDs). The distance between groups 1 and 3 is 44.7 SDs. Finally, the distance between groups 2 and 3 is 63.6 SDs. The variations accounted for by each of the first six principal components and the cumulative variation for the first six principal components of the complete dantrolene library are given in Table 2.

Table 2. Variation accounted for by each of the 6 largest principal components of the spectra of the dantrolene library.

PC Number	Variation in this PC	Cumulative PC Variation
1	0.3256	0.3256
2	0.1737	0.4993
3	0.1062	0.6055
4	0.0571	0.6626
5	0.0480	0.7106
6	0.0292	0.7398

A scatterplot of PCs 4 through 6 of the dantrolene library appears in Figure 7. PCs 1 through 3, which show the three groups, together account for 61% of the total spectral variation. On the other hand, PCs 4 through 6, which do not show the three groups, account for only 13 percent of the total spectral variation.

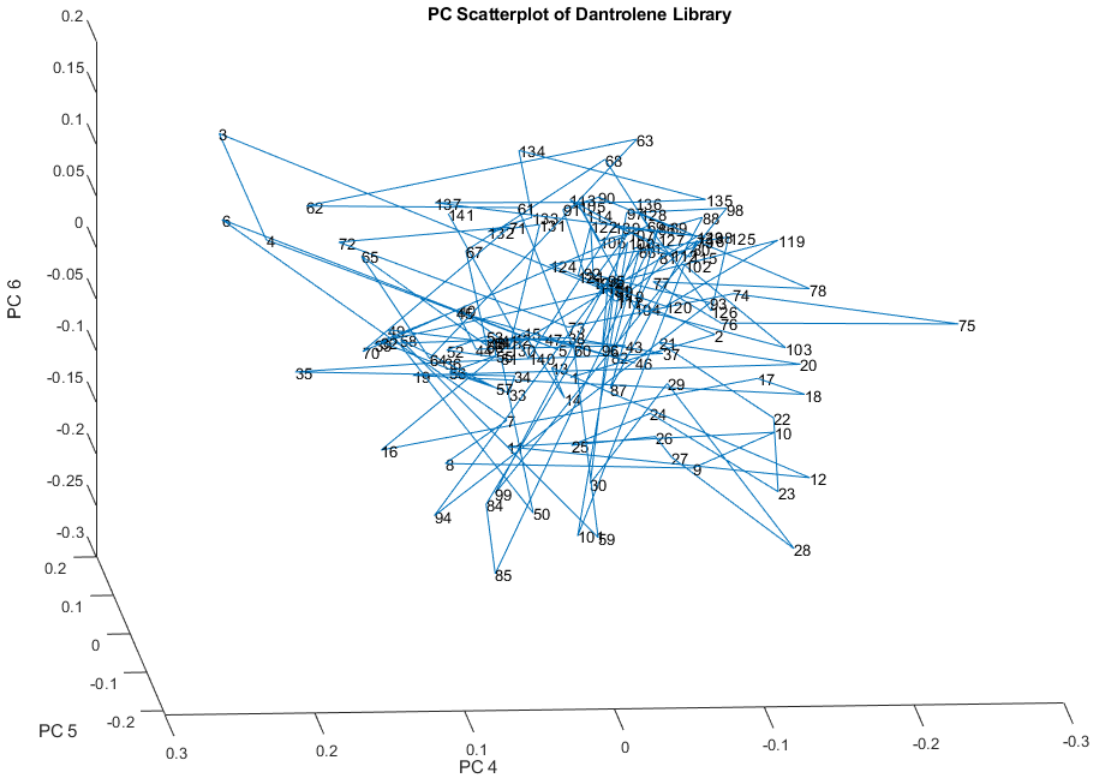

Figure 7. PC scatterplot of the 141 vials in the dantrolene library on PCs 4, 5, and 6. In this plot using smaller PCs than Figure 6, the three separate groups are not visible.

Figure 8 graphs the spectrum of the center points in each of the three groups shown in Figure 6. As one progresses from group 1 through group 3 in Figure 8, peaks at 4046, 4177, 4621, and 4779 cm^{-1} intensify. At the same time, peaks at about 4240 and 4425 cm^{-1} are diminished.

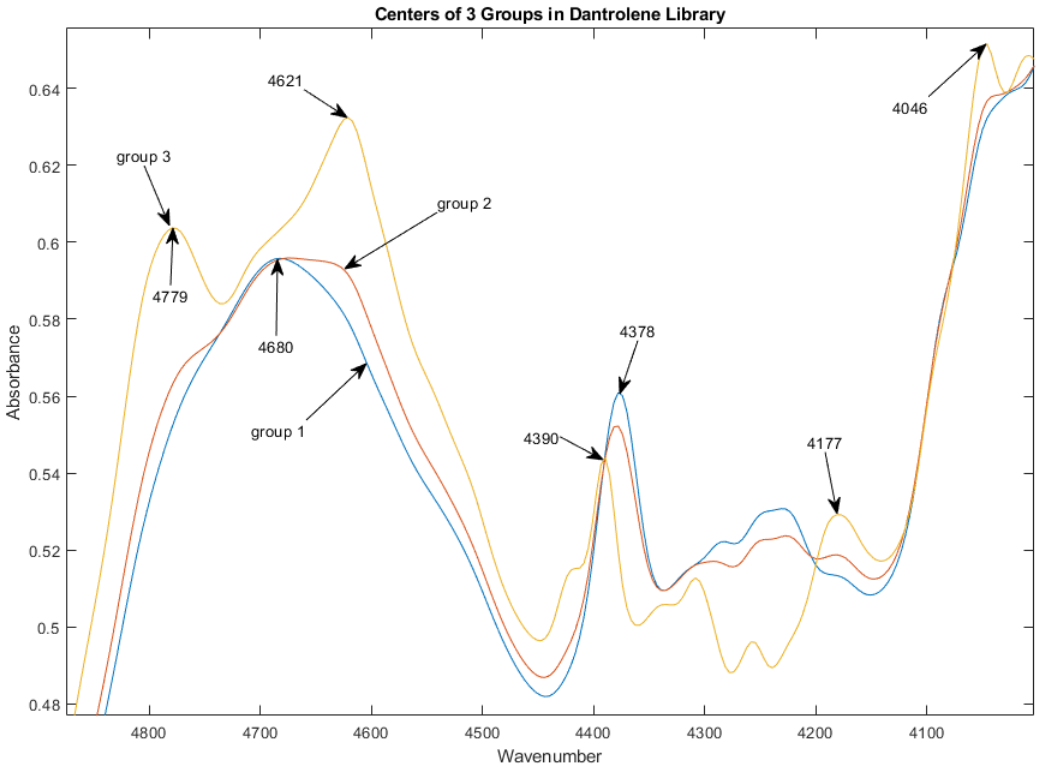

Figure 8. The spectrum of each of the centers of the three groups in Figure 6 (the groups can be identified as 1, 2, and 3, numbering from from left to right in Figure 6). Group 1 (blue line) contains 50 vials, group 2 (red line) contains 78 vials, and group 3 (yellow line) contains 13 vials.

Figure 9 depicts the loadings for PC1 of the spectra in the dantrolene library containing lots19REV07A, 19REV08A, and 19REV12A and 20REV01A. This loadings spectrum shows the effects of baseline variation following multiplicative scatter correction. Major peaks are marked at 4264, 4389, 4555, 5932, 8277, 8493, and 9721 cm^{-1}.

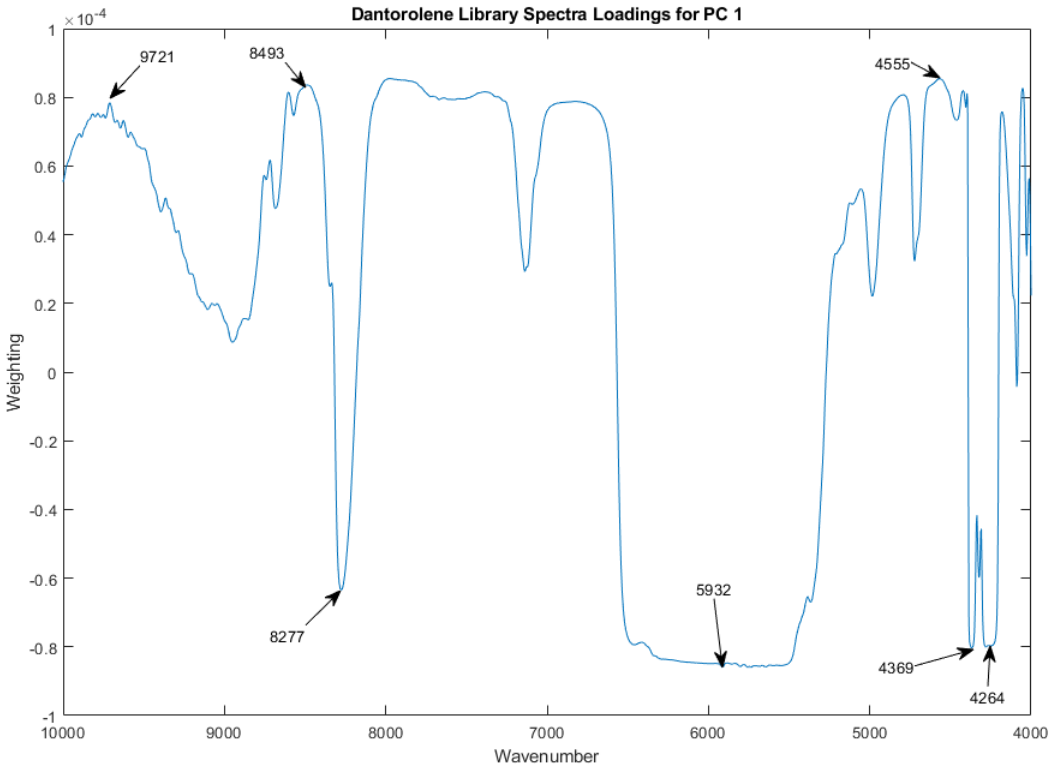

Figure 9. Loadings for PC1 of the spectra in the dantrolene library containing lots19REV07A, 19REV08A, and 19REV12A and 20REV01A. This loadings spectrum shows the effects of baseline variation following multiplicative scatter correction. Major peaks are marked at 4264, 4389, 4555, 5932, 8277, 8493, and 9721 cm^{-1}.

Figure 10 graphs the loadings for PC2 of the spectra in the dantrolene library. Major peaks are marked at 4391, 4972, 5269, 6587, 8180, and 8984 cm^{-1}.

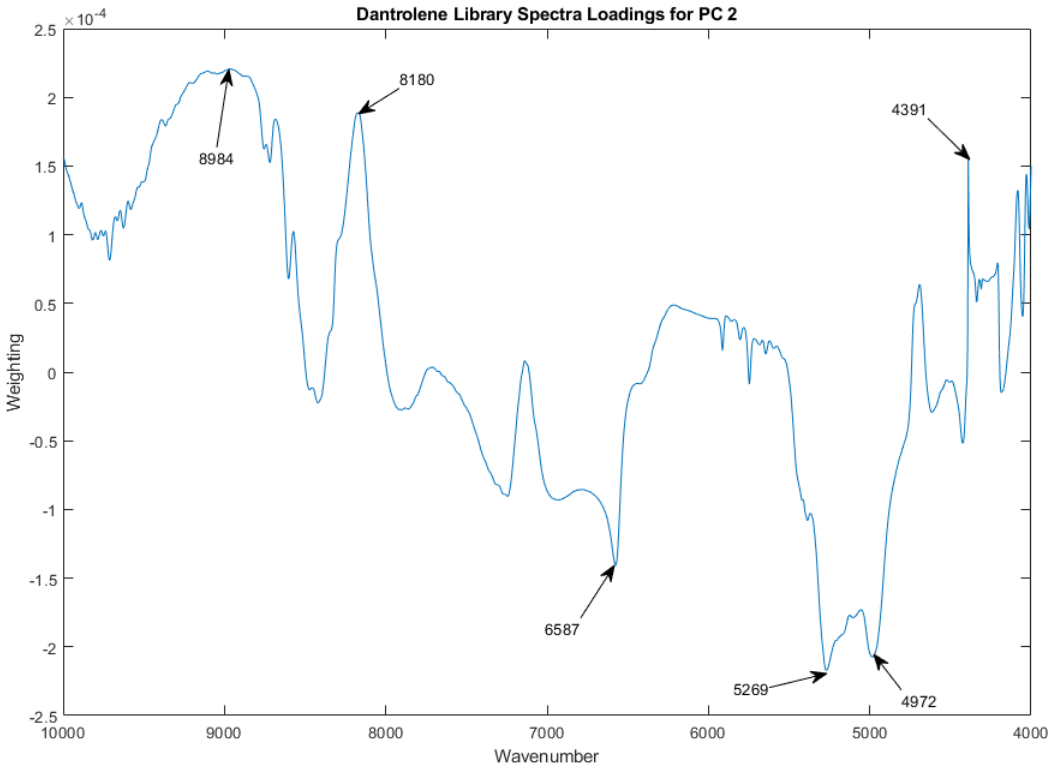

Figure 10. Loadings for PC2 of the spectra in the dantrolene library containing lots 19REV07A, 19REV08A, and 19REV12A and 20REV01A. Major peaks are marked at 4391, 4972, 5269, 6587, 8180, and 8984 cm^{-1}.

Figure 11 shows the loadings for PC3 of the spectra in the dantrolene library. Major peaks are marked at 4197, 4733, 6565, and 7138 cm^{-1}.

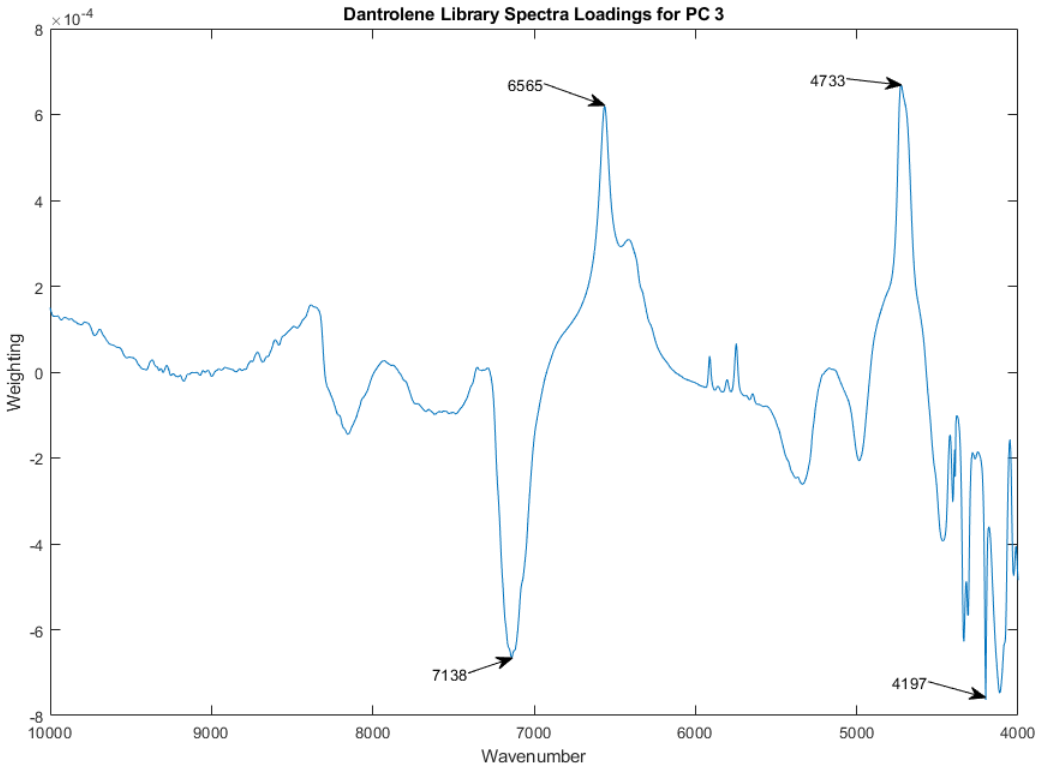

Figure 11. Loadings for PC3 of the spectra in the dantrolene library containing lots 19REV07A, 19REV08A, and 19REV12A and 20REV01A. Major peaks are marked at 4197, 4733, 6565, and 7138 cm^{-1}.

Conclusions

Dantrolene is a muscle relaxant, orange powder, used to combat malignant hyperthermia. It can be taken orally in capsules of 25, 50, or 100 mg, or it can be injected intravenously. Depending on the manufacturer, dantrolene may also contain mannitol, polysorbate 80, and povidone K12 (Kleinman et al., 2021).

The Drug Quality Study (DQS) used Fourier transform near-infrared spectrometry (FTNIR) to measure the variability of dantrolene spectra within and between lots. Samples from lot 20REV01A fell into two groups that were 66.7 standard deviations apart, suggesting that the two groups contained different materials and may have been manufactured differently. As a result, the entire library of four lots of dantrolene spectra was analyzed. The spectra of the 141 vials of

dantrolene in the library were found to contain 3 separate groups, each comprising different materials.

Uniformity and quality are essential for drug manufacturing. When either is lacking, it can lead to negative outcomes for patients and companies. This is especially important for dantrolene, a powerful drug that is only to be used in very specific situations. The results from FTNIR show that there may be problems with content uniformity in the materials and/or variability in the techniques used to manufacture dantrolene. Further investigation is needed.

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