Spectrometric Results of Process Variations in Dacarbazine

James T. Isaacs[1], Philip J. Almeter[1, 2], Bradley S. Henderson[1], Aaron N. Hunter[1], Thomas L. Platt[1], Robert A. Lodder[3,*]
University of Kentucky
Lexington, KY 40536

1. Department of Pharmacy Services, University of Kentucky, Lexington, KY 40536
2. Pharmacy Practice & Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506
3. Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536
*Author to whom correspondence should be addressed. Email: Lodder @ g.uky.edu

RAPID COMMUNICATION

Abstract

Intra-lot and inter-lot variability in dacarbazine was detected in the Drug Quality Study (DQS) using Fourier transform near-infrared spectrometry (FTNIR). One vial of six vials sampled from Fresenius Kabi Lot 6125612 appeared 7.8 SDs from the center of the rest of the vials on the DQS FTNIR screening assay. Spectra of 54 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

Dacarbazine is a colorless to ivory-colored solid that is sensitive to light. Dacarbazine is used in the treatment of metastatic malignant melanoma. In addition, it is also indicated for Hodgkin's disease as a second-line therapy when used in combination with other effective agents.

Issues

The DQS team has identified possible quality control issues (intra-lot and inter-lot variability) with dacarbazine 200 mg manufactured by Fresenius Kabi that may require further investigation.

The lot with intra-lot variability was Lot 6125612 with an expiration date of 01/23. The lots forming the spectral library were 6123591, 6124241, 6124355, 6125287, 6125431, and 6125612.

Methods

FTNIR (Fourier Transform Near-Infrared) Spectrometry

Using nondestructive analytical techniques, FTNIR spectra were collected for inventory belonging to Lot 6125612 as part of routine medication quality screening. A representative sample of 6 individual vials were selected for screening from Lot 6125612 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 1 of 6 vials screened after applying multiplicative signal correction to the spectra. In general, differences in absorbance peak location and intensity were observed at 6514, 6694, 6836, and 7081 cm^{-1}. These findings can be observed in Figures 1, 2, and 3.

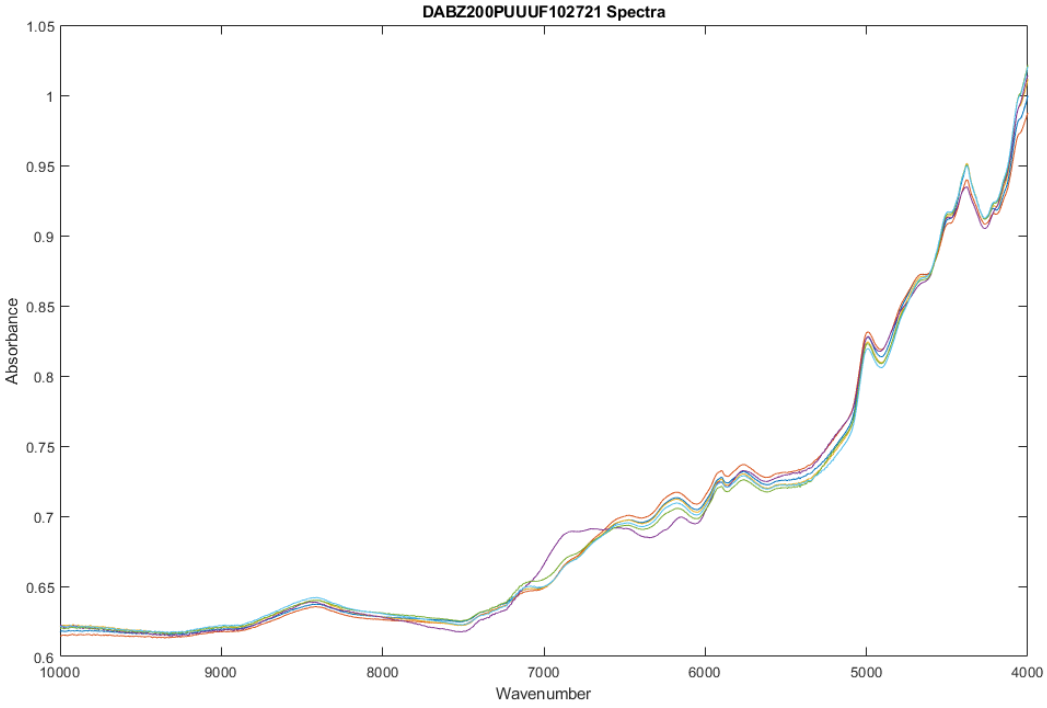

Figure 1. Spectra of 6 vials of dacarbazine from Lot 6125612 after multiplicative scatter correction. There is one apparent outlier (purple line, vial 4). The major peak differences appear around 6300 and 6836 cm^{-1}.

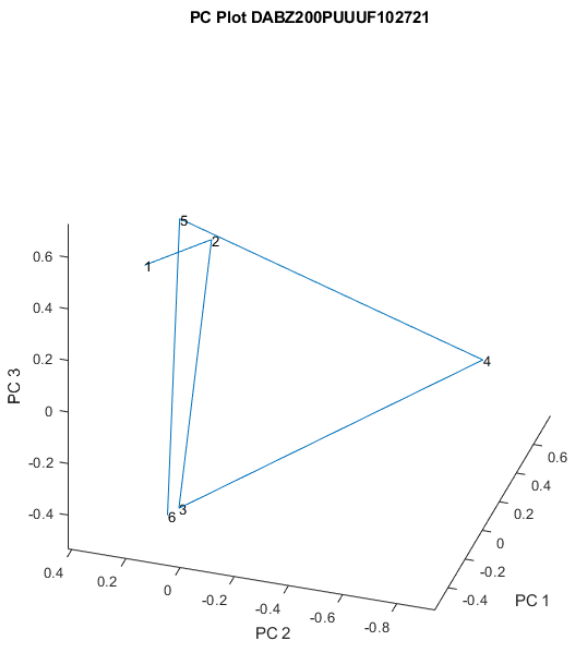

Figure 2. PC plot of 6 vials from Lot 6125612 after multiplicative scatter correction and z-scoring of the spectra. Vial 4 is 7.8 SDs from the mean of the others on PC 2.

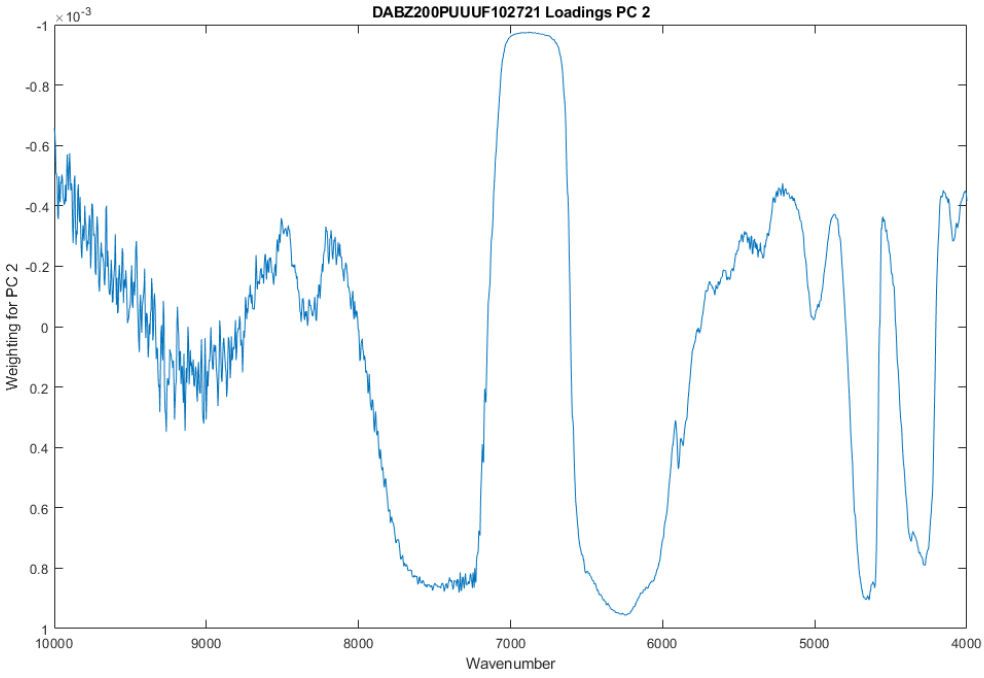

Figure 3. Loadings of PC 2 for Lot 6125612. The largest loadings with the largest absolute values represent spectral regions most important in forming PC 2.

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. Statistical analysis of the collected FTNIR spectra suggested intra-lot variability. The outlier vial (vial 4) on PC 2 had a calculated distance of 7.8 multidimensional standard deviations (SDs) from the center of the 5 other vials with the same lot number (see Figure 2). Using a group membership range between 0 and 3 SDs, a standard deviation of 7.8 signals a potential quality control issue in Lot 6125612.

Inter-Lot Analysis

In addition, upon comparing Lot 6125612 to other lots collected for the DQS spectral library (6123591, 6124241, 6124355, 6125287, and 6125431), it was discovered that there is inter-lot variability as well as intra-lot variability (see Figure 4). Figures 4 and 5 show that 6 vials are displaced from the main pair of vial clusters on PCs 2 and 3 (vials 2, 12, 58, 60, 64, and 67). Figure 6 is the spectra of 54 vials of dacarbazine from Lot 6125612 from 6200-7100 cm^{-1}. The displacement between the 2 clusters is statistically significant (p=0.02) using the subcluster detection test (r_{lim} = 0.98, r_t=0.86), with the QQ plot shown in Figure 7. If the two groups of vials were identical, the QQ plot would show a straight line with a slope of 1 and an intercept of zero.

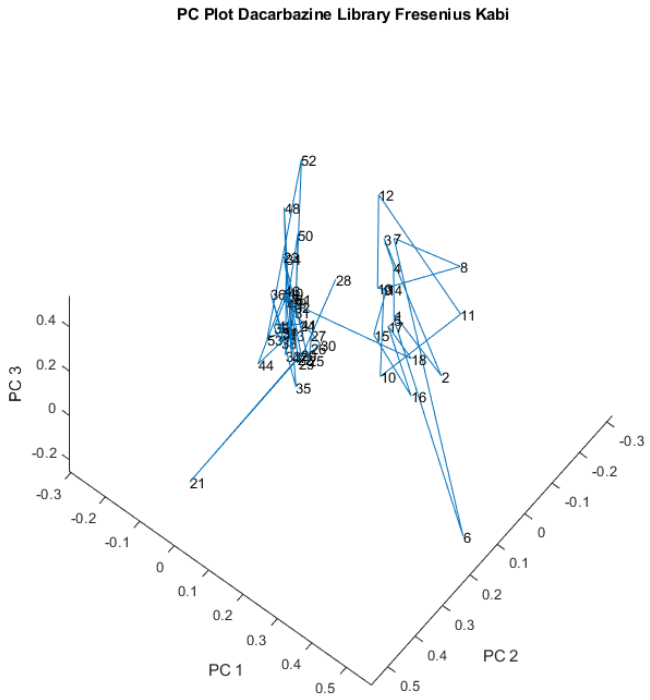

Figure 4. PC plot of dacarbazine spectral library. The dacarbazine spectral library (comprising Lots 6123591, 6124241, 6124355, 6125287, 6125431, and 6125612) clusters in 2 regions of hyperspace, with 8 suspected outliers (vials 6, 7, 8, 11, 21, 30, 44, and 52). Vials 6 and 21 stick out in this rotation.

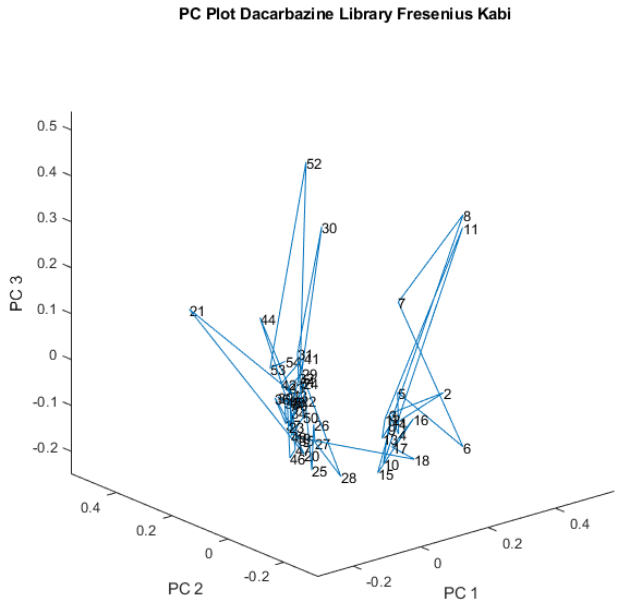

Figure 5. PC plot of dacarbazine spectral library. The dacarbazine spectral library (comprising Lots 6123591, 6124241, 6124355, 6125287, 6125431, and 6125612) clusters in 2 regions of hyperspace, with 8 suspected outliers (vials 6, 7, 8, 11, 21, 30, 44, and 52). Vials 7, 8, 11, 21, 30, 44, and 52 stick out in this rotation.

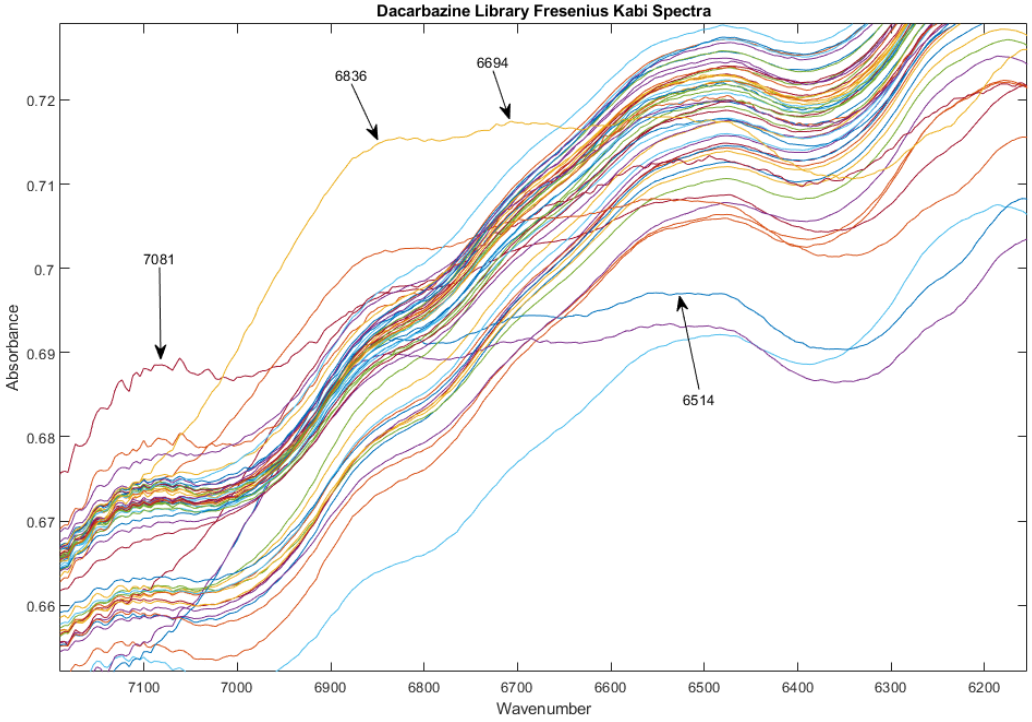

Figure 6. Spectra of 54 vials of dacarbazine from Lots 6123591, 6124241, 6124355, 6125287, 6125431 and 6125612 after multiplicative scatter correction. The wavenumbers of major peak differences are shown on the spectra.

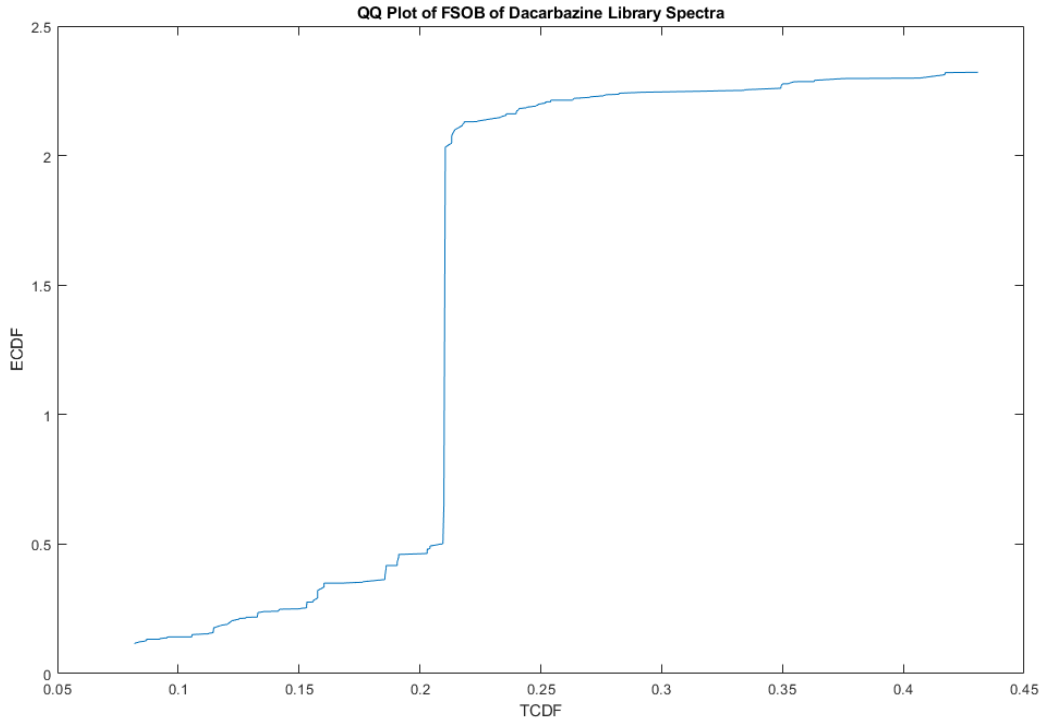

Figure 7. QQ plot from the subcluster detection method. This displacement between the groups of vials is statistically significant (p=0.02) using the subcluster detection test (r_{lim} = 0.98, r_t=0.86).

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 Fresenius Kabi dacarbazine 200 mg. One vial of six vials sampled from Fresenius Kabi Lot 6125612 appeared 7.8 SDs from the center of the rest of the vials on the DQS FTNIR screening assay. Spectra of 54 vials from six lots in the library clustered in two groups (p=0.02), suggesting they represent different material. Additional destructive testing should be conducted to determine whether these lots represent acceptable drug products.

Acknowledgements

The project described was supported in part by NSF ACI-1053575 allocation number BIO170011 and the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

Dempsey, R. J., Davis, D. G., Buice Jr, R. G., & Lodder, R. A. (1996). Biological and medical applications of near-infrared spectrometry. Applied Spectroscopy, 50(2), 18A-34A.

Isaksson, T., & Næs, T. (1988). The effect of multiplicative scatter correction (MSC) and linearity improvement in NIR spectroscopy. Applied Spectroscopy, 42(7), 1273-1284.

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: a review and recent developments. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 374(2065), 20150202.

Lodder, R. A., & Hieftje, G. M. (1988). Detection of subpopulations in near-infrared reflectance analysis. Applied spectroscopy, 42(8), 1500-1512.