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Evaluation of key sources of variability in the measurement of pharmaceutical drug products by near infrared reflectance spectroscopy

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Abstract

Potential sources of variability in the measurement of solid oral drug products by near infrared reflectance spectroscopy were evaluated with statistical experimental design. Spectra were collected for two different tablet types according to the data collection and treatment parameters defined by the experimental design. Each tablet had three different dose-levels. Libraries were constructed using second-derivative spectra. Key figures-of-merit generated during internal and external library validation were used to calculate which parameters most strongly influence the library performance for dose-level discrimination. These responses and their corresponding experimental conditions were evaluated with the screening model in the JMP® program. Segment value used for the second-derivative calculation was an influential factor and had a complex effect. Orientation on the sampling platform also had an influential effect for embossed tablets. Collection of spectra over fewer days decreased variability within the library. More frequent reference spectrum collection improved the performance of libraries to a small degree. A larger sample population increased the range of spectral variability within a dose-level but apparently not the overall performance of the library. The number of scans averaged per spectrum was not an influential factor in this study. These results are summarized and used to recommend an approach to dose-level discrimination.

Keywords: Near infrared; NIR; NIRA; NIRS; Reflectance spectroscopy; Clinical trial materials; Experimental design; Product discrimination; Identification test

1. Introduction

Near infrared reflectance spectroscopy (NIRS) is a rapid analysis technique that has grown in its use in the pharmaceutical industry [1–3]. Applications include water determination [4,5], identification [6–9], evaluation of mixing homogeneity [10], and quantitative quality control [11,12]. In all cases, the most important aspect of a successful measurement is the construction of an appropriate calibration set and the control or inclusion of all significant sources of variability.

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A primary goal in the authors' laboratory has been to understand the parameters that affect the ability to use NIRS to differentiate between dose-levels of drug products. As applied to final package identification, this testing is one aspect of clinical material analysis in the pharmaceutical industry. Feasibility studies have appeared in the literature [7,8] but little work has been presented on the effect of data collection and treatment parameters on this type of measurement. The work presented here provides a scientifically based model upon which the authors can recommend operating conditions for good sensitivity and efficiency. In daily operation, analysts must discriminate between low and closely spaced dose-levels, making this information valuable.

In this study, statistical experimental designs [13] were used to evaluate how selected sources of variability impact the quality of a spectral library built for dose-level discrimination. Parameters included instrument settings such as the number of scans averaged per spectrum, data treatment settings such as the segment used for second-derivative calculation, and the design of the library such as the number of dose units scanned. Plackett–Burman designs were generated that defined a series of experiments using different settings of these parameters. Responses from each experiment were chosen that correlated to the ability of the library to separate between dose-levels. In general terms, a better library is one where the spectra for a given dosage level are tightly grouped and the separation between the mean spectra of different dosage levels is the largest. Responses were chosen to highlight these traits. This approach allowed the evaluation of many parameters under actual conditions of the desired measurement in a way that was not possible when doing experiments that only vary one parameter at a time.

2. Experimental

2.1. Materials and instrumentation

Typical tablets, A and B, under development at Eli Lilly and Company were used for this study. There were three dosage levels of tablet A (25, 50, and 75 mg drug per 200-mg tablet) and three dosage levels of tablet B (30, 60, and 150 mg drug per 250-mg tablet). Both tablets were oblong and tablet A was embossed on both sides. Tablet B was smooth on both sides. Three hundred tablets of each type were used.

Diffuse reflectance spectra of these tablets were obtained with an NIRSystems 6500 spectrophotometer (Foss-NIRSystems, Silver Springs, MD) equipped with a Rapid Content Analyzer (RCA). The RCA has a horizontal glass platform and an iris aperture that centers the tablet in the path of the light beam. The incident radiation comes from below and an array of six lead sulfide detectors is also aligned below the glass sample platform (for graphic description, see Ref. [7]). Each spectrum was the average of 8, 32, or 64 scans over the range of 1100–2500 nm. All spectra were log ratioed against a reference sample of white ceramic. Software packages NSAS ver. 3.50 and IQ^2 ver. 1.20, accompanying this instrument, were used to build and validate libraries. Libraries were built with second-derivative spectra, which is a common treatment for baseline shift and particle-size effects.

2.2. Experimental design

Many experimental factors contribute to variability when collecting and analyzing NIRS spectra of solid oral drug products. We chose the following factors to include in this study:

1. *Iris*. The iris is an aperture used to center samples on the platform of the RCA. Although it is designed to be left open during data collecting, it can be set at various positions between closed and wide open, affecting the level of scattered light.
2. *Segment*. This is the number of data points over which slope is calculated during the conversion to second-derivative spectra and is a smoothing function. A low segment value will fail to smooth out noise. On the other hand, spectral information related to the analyte of interest is lost if the segment value is set too high.

Table 1
Experimental design with seven factors and four center points for Tablet A

Run	Iris	Segment	Orientation	Scans	Samples	Days	Frequency
1	Half-way	8	Fixed	32	30	3	16 min
2	Open	10	Fixed	16	15	1	30 min
3	Open	5	Fixed	64	45	1	2 min
4	Open	5	Varied	16	15	5	2 min
5	Closed	10	Varied	16	45	1	2 min
6	Half-way	8	Fixed	32	30	3	16 min
7	Closed	5	Fixed	16	45	5	30 min
8	Open	10	Varied	64	45	5	30 min
9	Closed	10	Fixed	64	15	5	2 min
10	Half-way	8	Fixed	32	30	3	16 min
11	Closed	5	Varied	64	15	1	30 min
12	Half-way	8	Fixed	32	30	3	16 min

3. *Orientation*. This instrument has a limited number of detectors positioned to collect diffuse reflectance. The objective was to determine if varied orientation on the sample platform would introduce significant variability.
4. *Number of Scans* (Scans). If more scans are averaged per spectrum, more instrument variability should be averaged out. However, increased scans per spectrum makes data collection time longer and could be of minimal value for a low-noise instrument.
5. *Total Number of Samples* (Samples). A certain number of samples are needed to account for the physical and chemical variations between tablets. Including spectra of more tablets than necessary reduces efficiency.
6. *Number of Days* (Days). The number of days over which an experiment is performed could be another source of variability, especially if the performance of the instrument varies from day to day. The goal was to determine if a library could be built on a single day and still include sufficient variability to discriminate dose-levels in the future.
7. *Reference Frequency* (Frequency). Because the instrument is a single-beam configuration, it was necessary to collect a separate reference spectrum occasionally. The more often this is done, the less influence instrument drift will have, but data collection will take longer.

Examples of major factors not studied include lot-to-lot variability in manufacturing, within-lot homogeneity, and temperature.

Experimental designs were performed to investigate the main effects of the above possible sources of variability with the screening fit application of JMP® software (SAS, Cary, NC). This fitting platform is designed to analyze experimental data where there are many effects but few observations. A seven-factor, eight-run Plackett–Burman experimental design with four center-point runs was created for tablet A. This design is listed in Table 1 and only has enough resolution to determine if individual factors have some effect on the response and whether there is evidence of general curvature due to at least one of the factors. For tablet B, a six-factor, eight-run Plackett–Burman design with four center-point runs was generated and is listed in Table 2.

2.3. Spectrum collection

Spectra were collected for tablets A and B according to the conditions in Tables 1 and 2, respectively. In run four of Table 1, for example, three tablets were scanned on five different days. The instrument was set to average 16 scans, which takes about 20 s. Including time to position the sample and operate the software, it took about 2 min to collect three spectra, so only one reference

Table 2
Experimental design with six factors and four center points for Tablet B

Run	Segment	Orientation	Scans	Samples	Days	Frequency
1	10	Fixed	32	30	3	16 min
2	5	Varied	8	45	1	30 min
3	10	Fixed	32	30	3	16 min
4	5	Varied	64	15	5	2 min
5	20	Varied	64	45	5	30 min
6	20	Varied	8	15	1	2 min
7	20	Fixed	64	15	1	30 min
8	5	Fixed	8	15	5	30 min
9	10	Fixed	32	30	3	16 min
10	5	Fixed	64	45	1	2 min
11	20	Fixed	8	45	5	2 min
12	10	Varied	32	30	3	16 min

scan was needed prior to data collection. The tablets were centered but randomly oriented including varying which side was presented to the light source and the rotation angle with respect to the detectors. The iris was used to center the tablets and left open during data collection. Finally, a segment of five was used to calculate the second-derivative spectra. Note that ten external validation tablets of each dose-level were scanned on a different day according to the conditions of each run.

2.4. Library validation

Libraries were built using the IQ^2 software using the 'distance' calculation for spectral comparison. Distance is the largest difference between spectra in standard deviation units at any individual wavelength. Each dose-level is considered a separate 'product' and a mean spectrum is calculated for each product. The software then calculates a distance value for each individual spectrum from the mean. The distance of individual product spectra from their mean is a measure of the amount of variability in a group. The distance from a mean spectrum to a spectrum of a different product is a measure of separation between dose-levels.

3. Results and discussion

3.1. Spectral regions

Proper dose-level discrimination depends on the selection of spectral region that corresponds to absorption bands of the active drug substance. In the NIR region, most absorption bands arise from the overtone and combination bands of the fundamental infrared vibrations of C–H, N–H, and O–H bonds.

Significant changes of NIR spectral features of these tablets have been observed when dose-level varies. For example, as the active ingredient of tablet B increased, the absorption band of the stretching vibration in the mid-IR at 3145 cm^{-1} (3.18 μm) increased accordingly and to a maximum in the case of pure active ingredient. Because the second overtones and/or combination bands appearing around 1130 nm originated from these stretching vibrations, they reflected the existence and amount of active ingredient of tablet B. Accordingly, there are significant differences for different dose-levels of tablet B in the spectral region of 1110–1150 nm (see Fig. 1). Similar studies were done to verify that spectral differences in the region of 1130–1180 nm between dose-levels of tablet A are largely due to the active ingredient (see Fig. 2).

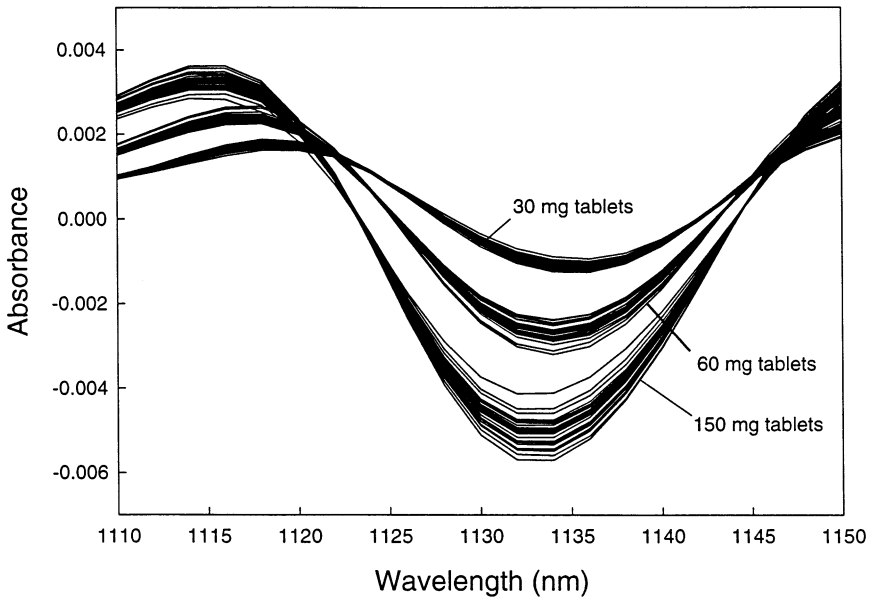

Fig. 1. Second-derivative NIR spectra of the different dosage strengths of tablet B in the spectral range of 1130–1180 nm from the fourth experimental run. The derivative was calculated with a segment of 5.

3.2. Response functions

Using the library constructed for each run and the external validation spectra, four responses were calculated. As mentioned, these responses are figures-of-merit that correlate to the quality of the library for dose-level discrimination. Also, it is expected that these responses correlate to the quality of the spectra for building a quantitative calibration model. Thus, the conclusions indirectly apply to the use of NIRS as an alternate quantitative technique.

Largest qualifying distance with no conflicting pairs. A conflicting pair occurs when the error bars on the mean spectrum of any two dose-levels overlap at all wavelengths. If at least one wavelength can be found where, for example, 4σ error bars do not overlap, there are no conflicting pairs at 4σ. The error interval was increased until the largest qualifying distance with no conflicting pairs (LQD) was found. This response was based on both the distance separating the products and the standard deviation within each product. The IQ^2 software uses this calculation as part of the internal library validation. The larger this value, the easier it is to discriminate between products. Therefore, it was a useful response to evaluate the libraries built by different experimental designs. The LQD values for different runs of tablet A are listed in Table 3 and vary from 5.6σ to 18.4σ. For tablet B, the LQD values for different runs are tabulated in Table 4 and vary from 7.8σ to 13.6σ.

Qualifying distance from the mean set. Qualifying distance from the mean set (QDFMS) was the distance of each individual spectrum from the mean spectrum of that dose-level. It could reflect both actual differences between samples and sources of variation in instrumentation and data treatment. It was correlated to how tightly the spectra of a given dosage level were grouped. The average of the five largest QDFMS (σ) values regardless of dose-level was reported in Tables 3 and 4. This response varied from 2.36σ to 3.81σ for tablet A and from 2.07σ to 3.71σ for tablet B.

Distance to the closest wrong set. Distance to the closest wrong set (DCW) was the distance of the spectrum of an external validation tablet to the closest wrong dose-level mean spectrum. The larger this value, the less the chance of this tablet being identified as the wrong dose-level. Its value

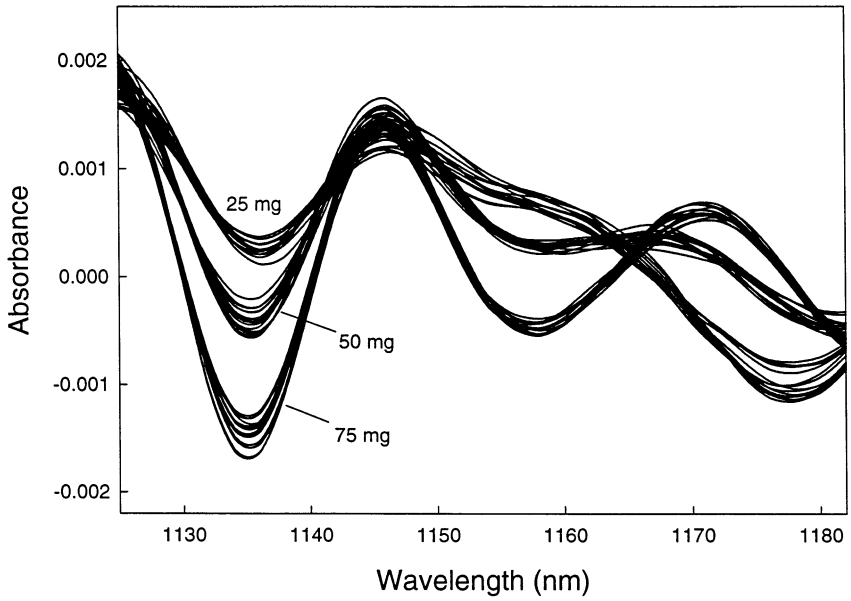

Fig. 2. Second-derivative NIR spectra of the different dosage strengths of tablet A in the spectral range of 1110–1150 nm from the second experimental run. The derivative was calculated with a segment of 5.

reflected the uniformity of the tablets in the same product and also the spectral separation between different products (similar to LQD). The DCW result is strongly affected by the selection of spectral region used for the calculation of the DCW. A spectral region corresponding to the main drug substance band was used in order to model sources of variability that impact dose-level discrimination. For tablet A this spectral region was 1130–1140 nm while spectral regions of 1110–1144, 1116–1145, and 1134–1148 nm were used for calculations with segment values of 5, 10, and 20 for tablet B, respectively. The DCW values in Tables 3 and 4 are for the 75-mg dose of tablet A and the 150-mg dose of tablet B. Each was calculated from the average of the three smallest DCW (σ) values from the ten external validation tablets. DCW varied from 12.36σ to 34.36σ and 26.43σ to 74.10σ for tablets A and B, respectively.

Table 3
Responses from library validation for Tablet A

Run	LQD (σ)	QDFMS (σ)	DCW (σ)	DLS (mg)
1	15.4	3.09	28.61	2.60
2	18.4	2.36	28.99	1.98
3	11.9	3.16	20.19	3.64
4	5.7	2.36	16.26	3.37
5	6.4	3.10	18.92	3.80
6	15.4	2.75	25.90	2.52
7	7.1	3.81	12.36	7.28
8	5.8	3.20	18.95	3.95
9	8.4	2.65	34.36	1.83
10	13.1	3.28	29.66	2.66
11	5.6	2.50	13.70	4.24
12	14.6	2.77	31.28	2.13

Table 4
Responses from library validation for Tablet B

Run	LQD (σ)	QDFMS (σ)	DCW (σ)	DLS (mg)
1	13.6	3.12	63.52	4.18
2	9.5	3.62	38.44	8.08
3	11.5	3.18	52.70	5.12
4	7.8	2.72	36.40	6.38
5	9.9	3.71	40.07	7.67
6	12.3	2.56	74.10	2.86
7	9.9	2.65	61.70	3.64
8	8.3	2.65	26.43	8.66
9	10.3	2.07	43.29	4.08
10	10.8	3.43	58.52	5.14
11	10.0	3.63	46.92	6.39
12	11.7	2.96	52.48	4.79

Table 5
Evaluation of experimental design for Tablet A

Response	Major factors	*P*-value	Preference
LQD	Orientation	0.01	Fixed
	Days	0.02	Less days
	Iris	0.02	Open
	Segment	0.07	High
DCW	Segment	0.01	High
	Orientation	0.02	Fixed
	Samples	0.04	Less samples
QDFMS	Samples	0.02	Less samples

Table 6
Evaluation of experimental design for Tablet B

Response	Major factors	*P*-value	Preference
LQD	Curvature	0.03	Middle
	Days	0.07	Less days
	Segment	0.10	High
DCW	Days	0.01	Less days
	Segment	0.03	High
	Frequency	0.09	Less minutes
QDFMS	Samples	0.04	Less samples

Dose-level separation. QDFMS was approximately converted from standard deviation units to milligram units. This converted response was termed dose-level separation (DLS) and DLS values are also listed in Tables 3 and 4. In the case of tablet B, for example, DLS values were calculated by first dividing the difference in dose between the 150- and 60-mg tablets by the DCW value for each run. This was essentially a slope value of mg/σ. This slope was then multiplied by the corresponding QDFMS value for that run to give milligram units. The values of DLS more clearly illustrate how different the most deviating spectrum is from the mean spectrum of a product under the different experiment conditions. The value of DLS is correlated to the closest spacing of dose-levels that might be discriminated with each library. Note that the DLS response was not used as a response in the screening fit calculation because it was simply derived from other response functions. It is presented here because of its intuitive value.

3.3. Evaluation of sources of variation

The results of the two experimental designs were calculated with the screening fit calculation and key results are listed in Tables 5 and 6 for tablet A and tablet B, respectively. For each response the major influential factors (or parameters) were evaluated and selected based on the performance of fit and the *P*-value. The *P*-value represented the probability of getting an even greater *t*-statistic assuming the parameter is zero. *P*-values less than or close to 0.05 indicated that a change from the low to high setting of this factor had a significant effect on the response.

A complete set of parameter estimates of this calculation has been illustrated in Table 7 where each parameter was evaluated with LQD as the response. The estimate corresponded to the coefficient of the linear model found by least squares while the standard error (std error) was the standard deviation of the distribution of parameter estimate. The corresponding prediction profile of this calculation is presented in Fig. 3. It clearly indicated how each parameter influenced the LQD and thus the performance of the libraries. Error bars corresponded to the standard errors.

Following is a summary of results for each parameter. Specific results for tablets A and B are covered as well as the general implication for dose-level discrimination.

Table 7
Parameter estimation for experimental design of Tablet A with LQD as the response function

Parameter	Estimate	Std. Error	*P*-value	Importance
Iris	1.79	0.38	0.019	Yes
Segment	1.09	0.38	0.066	Yes
Scans	−0.74	0.38	0.150	No
Samples	−0.86	0.38	0.110	No
Days	−1.91	0.38	0.016	Yes
Frequency	0.56	0.38	0.239	No
Curvature	−1.84	0.87	0.123	No
Orientation	2.79	0.38	0.005	Yes

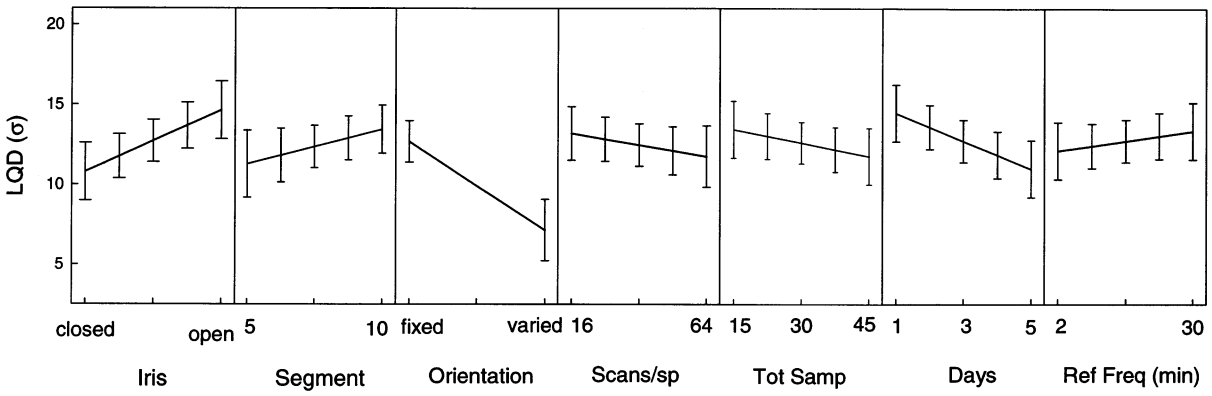

Fig. 3. Prediction profile for LQD at different parameter values for the experimental design of tablet A.

Iris aperture. The iris aperture was unique to this instrument and was not used for adjusting the light beam to the sample. Rather its intended function was to center the tablets on the sample platform. However, leaving this aperture closed (touching the edge of the tablet) should affect the level of scattered light. The iris aperture did influence the performance of the libraries. When it was open, better performance of the libraries was observed. When closed, the performance suffered, presumably due to increased scattered light.

Segment value. For both tablets A and B (see Tables 5 and 6), using larger segment values within the range studied resulted in better performance of the libraries. However, in the experiment for tablet A (Table 1), a segment of 20 resulted in complete loss spectral differences between doses. For tablet B, an increased range of segment values was possible. For tablet A, the LQD increased about 2.2σ (see Fig. 3) when the segment was changed from 5 to 10.

Further independent study of the influence of segment revealed that segment exerted a significant curvature influence. Additional libraries were built for each run with different segment values (5, 10, 12, 16, 20, 25, and 30) for tablet B. In Fig. 4, the average LQD values for runs with the same number of days were plotted against the segment values used. In all cases, there was a general increase when segment changed from 5 to 20. However, there was a maximum LQD at segment 20–25 when the library spectra were collected in 1 day or 3 days. The maximum LQD shifted to segment 10 when the library spectra were collected over 5 days. There seemed to be an interaction between segment and number of days. In practical applications, most libraries were built over several days and an optimum segment was easily obtained because it could be optimized with no effect on laboratory efficiency.

Orientation. Orientation was a major influential factor in the case of tablet A but not for tablet B. This difference was likely caused by the different appearance of these tablets. Tablet A was embossed on both sides while tablet B was smooth. Therefore, reflectance spectra of embossed tablets appeared to be more sensitive to orientation for this instrument design. The resulting libraries performed better with the orientation fixed than with the orientation varied for tablet A.

Scans. Results indicated that the number of scans averaged for each spectrum was not an influential factor for the selected responses. This result indicated that libraries can be built with spectra from a small number of scans, saving time without sacrificing performance.

Total samples. The QDFMS was most sensitive to the number of samples used because a larger number of tablets will bring in more variation within a dose-level due to imperfect uniformity and simple statistical sampling. There was also some influence on DCW. The total number of samples did not significantly influence LQD. In actual practice, the authors use a number of

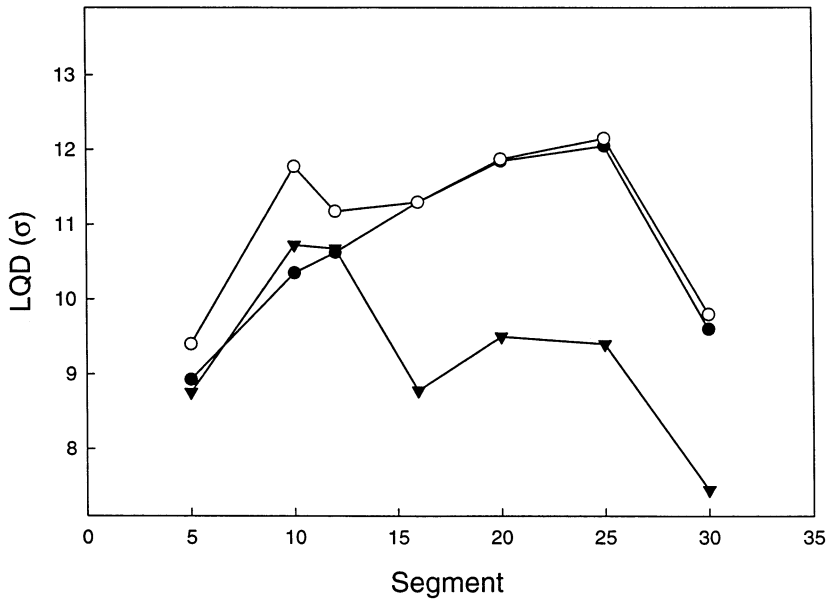

Fig. 4. Largest qualifying distance with no conflicting pairs versus the segment values of each corresponding library built in different numbers of days (1 day: -●-, 3 days: -○-, and 5 days: -▼-) for tablet B.

tablets for each new drug product that incorporates 95% of the population variation by using the NSAS sample selection procedure.

Days of spectra collection. The screening fit results indicated that building libraries over fewer days provided better response values. This makes sense in that spectra collected over a longer period of time would incorporate more variation due to factors such as instrument drift. Because the number of days is a factor that cannot be controlled, libraries must be built over multiple days for maximum robustness. However, if dose levels are spaced widely enough, libraries could be built in one day.

Reference frequency. The results of screening fit calculation for the influence of reference frequency were somewhat biased because of the interaction between number of samples, number of scans, and reference frequency. During an experiment the spectra were collected consecutively and a reference was taken whenever the experimental design required. As a result, when the number of samples was small and/or the number of scans was small, the data collection would be completed before the reference time interval. However, shorter reference intervals still corresponded to more frequent reference scans. Frequent collection of the reference during the spectrum collection yielded better results (based on the DCW response) for the libraries of both tablets, though this influence was slight. For typical dose-level separation, the interval between references could be as large as 30 min.

Curvature. A curvature factor was introduced to assess whether there was evidence of a substantially higher or lower response for runs at the middle levels of the factors as compared to those at the extremes. No strong evidence of curvature was observed for the responses measured for tablet A. In the case of tablet B, curvature was a significant factor for LQD, indicating that significantly different (better, in this case) average responses were observed at the middle level trials when compared to the extreme level trials. As discussed before, independent study of the effect of segment indicated that this curvature was most likely the cause (see Fig. 3).

As a simple verification of the statistical models, it was possible to empirically compare the results (Tables 5 and 6) to the responses (Tables 3

and 4). For example, without taking into account practical considerations presented above (e.g. building a library over multiple days was preferred), one would select open iris, fixed orientation, one day, and a segment of 10 with the rest parameters not restricted for tablet A. In Table 3, Run 2 corresponds to these conditions and provided the best responses. For tablet B, one would choose the conditions of Run 6 (one day and segment of 20 with other parameters not restricted). Similarly, Run 6 resulted in good responses (see Table 4).

4. Conclusion

This work has shown the influence and significance of various factors on the ability of NIRS to discriminate between dose-levels of tablets. Segment value was a significant factor with good results achieved by using a segment value of 10 when the libraries were built from spectra collected over several days. Orientation was a significant factor only for embossed tablets. Total number of samples was a major factor when focusing the response on uniformity between doses (QDFMS) but was not a significant factor for overall library performance. In addition, the number of scans per spectrum and reference frequency were not influential factors. This work supported that the iris aperture of this instrument should be left open during data collection. Finally, because the number of days was a significant source of variability but cannot be controlled, it was recommended that libraries be built over multiple days.

As mentioned above, sources of variability must either be controlled or built into the library. Typically investigators do one or the other for every conceivable source of variability. Based on this study, robust libraries can be built by controlling or incorporating only the appropriate sources of variability resulting in maximum efficiency. For example, Run 2 on Table 1 took about 25 min for library data collection while Run 3 (representing an attempt to build in maximum variability of scans, sample sizes and reference frequency) took about 9 h. However, both runs provide adequate libraries for dose-level discrimination of tablet A.

Understandably, these results apply best to this specific instrument configuration. However, several concepts presented here apply to any NIRS measurement, and this experimental design has great value to others doing similar work. Many pharmaceutical companies do use Foss NIRSystems brand equipment, and the results will apply directly.

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References

[1] E.W. Ciurczak, Appl. Spectrosc. Rev. 23 (1987) 147–163.
[2] J.D. Kirsch, J.K. Drennen, Appl. Spectrosc. Rev. 30 (1995) 139–174.
[3] K.M. Morisseau, C.T. Rhodes, Drug Dev. Ind. Pharm. 21 (1995) 1071–1090.
[4] I.R. Last, K.A. Prebble, J. Pharm. Biomed. Anal. 11 (1993) 1071–1076.
[5] R.G. Buice Jr., T.B. Gold, R.A. Lodder, G.A. Digenis, Pharm. Res. 12 (1995) 161–163.
[6] R.A. Lodder, G.M. Hieftje, Appl. Spectrosc. 42 (1988) 556–558.
[7] P.K. Aldridge, R.F. Mushinsky, M.M. Andino, C.L. Evens, Appl. Spectrosc. 48 (1994) 1272–1276.
[8] M.A. Dempster, J.A. Jones, I.R. Last, B.F. MacDonald, K.A. Prebble, J. Pharm. Biomed. Anal. 11 (1993) 1087–1092.
[9] F. Gonzalez, R. Pous, J. Pharm. Biomed. Anal. 13 (1995) 419–423.
[10] W. Plugge, C. Van Der Vlies, J. Pharm. Biomed. Anal. 11 (1993) 435–442.
[11] P. Corti, L. Savini, E. Dreassi, S. Petriconi, R. Genga, L. Montecchi, S. Lonardi, Proc. Cont. Qual. 2 (1992) 131–142.
[12] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. De La Pezuela, Talanta 40 (1993) 1671–1676.
[13] SAS Institute Inc., JMP® Statistics and Graphics Guide, Ver. 3.1, SAS Institute Inc., Cary, NC, 1995, pp. 176–195.