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Applications of NIR in early stage formulation development Part I. Semi-quantitative blend uniformity and content uniformity analyses by reflectance NIR without calibration models

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Abstract

This article describes a semi-quantitative reflectance near infrared (SQ-NIR) method for blend uniformity (BU) and content uniformity (CU) analyses in early stage formulation development. Applicability of the method depends upon three factors: separation of NIR signals of the active pharmaceutical ingredient (API) from placebo; strength of the signal; and a quantitative relationship between API concentration and NIR signal. Based on these three criteria, suitable NIR signals of the API, separated from those of placebo through suitable pretreatment of the spectra, can be used for BU and CU calculations without calibration models. The method was applied to an early stage formulation development project. Multiple batches of tablets were prepared and analyzed using the SQ-NIR method and a validated UV–VIS reference method. The SQ-NIR method was able to distinguish between batches that had satisfactory and unsatisfactory content uniformity and potency. In addition, effects of compression force and API particle size on the SQ-NIR results are discussed. It is proposed that the SQ-NIR method may be used as an independent test in early stage formulation development. The advantages and limitations of the method compared with traditional HPLC or UV–VIS methods are also discussed.

Keywords: Near infrared spectroscopy; Semi-quantitative; Reflectance; Blend uniformity; Content uniformity

1. Introduction

Rapid and non-destructive analysis of solid dosage forms by near infrared (NIR) spectroscopy is gaining popularity in pharmaceutical development. The first report of intact solid dosage form analysis by NIR was for the detection of adulterated over-the-counter drugs in the 1980s (Lodder et al., 1987). Since then, a large number of publications have demonstrated that NIR is applicable for assay, content uniformity (CU), and other testing of tablets and capsules (Gottfries et al., 1996; Ritchie et al., 2002; Wargo and Drennen, 1996). It has also been demonstrated that NIR methods for tablets and capsules could be validated according to the method validation guidelines issued by the FDA and International Conference on Harmonization (ICH, 1994, 1996). Accuracy, precision, repeatability and intermediate precision of the NIR methods were comparable with HPLC or other reference methods (Ritchie et al., 2002; Blanco et al., 2000; Bodson et al., 2006).

For NIR analysis of solid dosage forms, the commercially available instruments are designed to operate in two different modes: reflectance and transmittance. In reflectance mode, the instrument detects the diffused reflectance energy of the NIR radiation from the sample. Usually penetration depth of the NIR radiation is less than 1 mm. Therefore, only a portion of the sample is analyzed in this mode. This has important implications in interpretation of the results, which is discussed in this article.

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In reflectance mode, the tested sample is not limited by sizes and shapes. The NIR spectrum is obtained in full range (up to 2500 nm) with good signal to noise ratio. In transmittance mode, the NIR radiation that penetrates through the sample is measured. Therefore, a larger portion of the sample (but usually not the entire sample) is analyzed compared with reflectance mode. This usually results in better accuracy of the method (Gottfries et al., 1996). On the other hand, due to the larger sample thickness, the NIR spectrum in the combination band region becomes too noisy to be useful for quantitative analysis. NIR methods have been developed using both reflectance and transmittance modes (Ritchie et al., 2002; Blanco et al., 2000).

Regardless of the modes of operation, currently developed NIR methods require labor-intensive calibration models. Even qualitative analysis by NIR (e.g., identification test), which is often accomplished using the pattern recognition algorithms, requires establishment of calibration models (Blanco et al., 2000). In quantitative analysis, preparation and selection of the calibration set for model development and validation are of paramount importance. Separate calibration sets are usually needed for uniquely different formulations. Validated NIR methods may become unstable or unusable because of adjustments to the formulation, changes in physical properties of the API, or changes in the manufacturing unit operations. Blanco and Alcala (2006) summarized the approaches for preparation of calibration samples for tablet analysis by NIR. The above-mentioned factors related to calibration models have limited the use of NIR in early stage formulation development. To avoid these problems, we explored the feasibility of blend uniformity (BU) and CU tests by NIR without calibration models. The semi-quantitative NIR (SQ-NIR) approach is based on the assumption that homogeneity of powder blends and tablets can be evaluated based on the relative intensity change of properly selected NIR signals. In this article, we explore the feasibility of this semi-quantitative approach; define boundaries of the applications; and compare testing results obtained using SQ-NIR and a reference method. Test materials include both immediate release (IR) and controlled release (CR) formulations employing API of the same chemical and physical characteristics.

2. Experimental

2.1. Materials

Magnesium stearate was purchased from Merck KGaA (Darmstadt, Germany). Hydroxypropyl methylcellulose (HPMC) with a commercial name of METOLOSE SR 90SH-100000SR was from ShinEtsu (Tokyo, Japan) and supplied by Biddle Sawyer Co. (New York, NY). This study involves a proprietary API under development by the Johnson & Johnson Pharmaceutical Research & Development, LLC (JJPRD).

2.2. NIR instrument

A FOSS XDS near-infrared rapid content analyzer (RCA) was used for the sample analysis (FOSS NIR Systems, Laurel, MD, USA). Following are the NIR conditions:

Sampling module—rapid module without spot size
Detection/detector—reflectance/lead sulfide + silicon
Number of scans—32
Resolution—0.5 nm
Wavelength range—400–2500 nm
Math treatment—1st derivative (S. Golay)
Software—Vision (FOSS) and the Unscrambler (Camo Process AS, Nedre Vollgate, Norway; version 9.5)

The tablet samples were scanned directly without any preparation. The powder samples were placed in separate 20-mL scintillation vials and scanned through the bottom of the vials.

2.3. Preparation of immediate release tablets

The dose proportional tablets for the API were prepared through a wet granulation process, which included four steps: (1) fluidized bed granulation of API and excipients (except magnesium stearate), (2) milling, (3) blending and (4) compression. The API and excipients were granulated in a top spray fluidized bed granulator Glatt GPCG 15. The binder solution used was 10% Povidone (PVP) which was atomized through a nozzle at 2 bar. The dried granulation was milled by a Quadro Comil (Quadro, Inc. Millburn, NJ) with a 0.093 in. round opening screen at medium rotating speed. Before tablet compression, the granulation was mixed with magnesium stearate for 3 min in a 40 L Bohle bin blender (LB Bohle, Warminster, PA, USA) at 25 rpm. The tablets were compressed by using a Fette 1200 compression machine (FetteAmerica, Rockaway, NJ).

2.4. Preparation of controlled release tablets

The controlled release tablets for the API were prepared through a direct compression process. The API, HPMC and other excipients were blended in a 40 L Bohle bin blender for 10 min at 25 rpm. Magnesium stearate was then added and the powder mixture was blended for 5 more minutes. Tablets were manufactured by a rotary tablet press Manesty Beta Press (Thomas Engineering, Hoffman Estates, IL) with 7 mm × 17 mm capsule shaped tooling at the speed of 40 rpm.

2.5. UV reference procedure

The following UV spectrophotometric procedure was used to analyze the samples. The samples were prepared by adding a single tablet to 900 mL of 0.1N HCl and then mixing until dissolved. The samples were then analyzed at 273 nm using an Agilent 8453 UV–VIS spectroscopy system (Wilmington, Delaware, USA) with a 10 mm cell path lengths. The method was validated according to the ICH guidelines (ICH, 1994, 1996) and used in stability and release testing of the tablets. Method precision was determined for this method by performing repeated measurements of the same sample solution 10 times. Relative standard deviation (R.S.D.) of the results was <1.0%.

3. Results and discussion

Development of an API into marketable products requires lengthy and costly formulation development work. Particularly in the early stage of formulation development, the formulators may need to screen numerous formulations. For this particular compound, two different types of formulations are desirable, a dose proportional IR formulation and CR formulation. The formulators have the option to choose wet/dry granulation or direct compression process. Selection of the best formulations will have significant impact on the product's success, as well as cost of manufacturing. NIR can be a valuable tool in shortening development time by providing BU and CU results quickly.

3.1. Feasibility of SQ-NIR analysis without calibration models

In exploring feasibility of BU and CU analyses by SQ-NIR, the following three factors should be considered: (1) separation of the API signals from placebo; (2) intensity of the API signals; (3) a quantitative relationship between API concentration and the NIR signals. Fig. 1a shows the raw NIR spectra of a drug substance sample (red), a placebo of the IR formulations (blue) and an IR tablet sample (green) whereas Fig. 1b presents the corresponding first derivative spectra of the same samples. In the spectral region of 2000–2200 nm, the raw spectrum of the placebo shows a broad band that is related to the ROH functional groups of the excipients. On the descending slope of the placebo band (2100–2200 nm), a very strong API band (with a maximum at 2140 nm) is present, which is related to the RNH_2 functional group of the API. After the first derivative treatment of the spectra, the API signal is well separated from the placebo (Fig. 1b). In addition, the API signals show excellent intensity. It is a good practice to check method precision to ensure applicability of the SQ-NIR method. In this case, the method precision was checked by performing repeated scans of a 50 mg strength CR tablet (8.32% API) 10 times without disturbing the sample. Relative standard deviation of the results was about 0.5%.

To justify use of the SQ-NIR method for BU and CU analyses, a quantitative relationship between intensity of the signal and

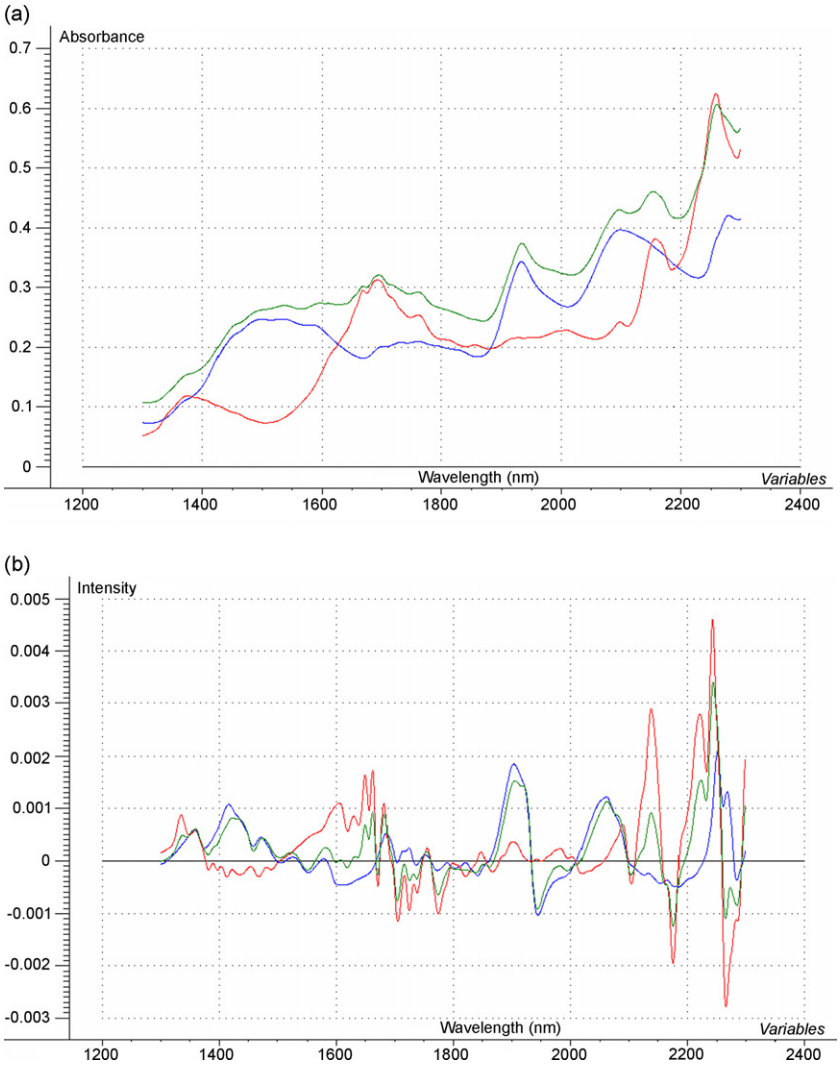

Fig. 1. Raw (a) and first derivative (b) spectra of the API (red), placebo (blue) and a tablet sample (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

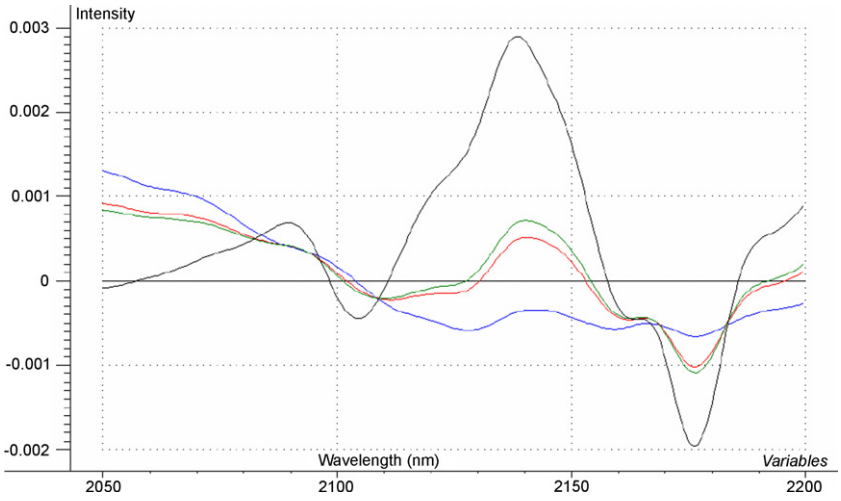

Fig. 2. Expanded first derivative spectra of the API (grey), 250 (green), 200 (red) and 50 mg (blue) CR tablets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

concentration of the API have to exist. In this case, CR tablets of three different strengths were selected and analyzed. Fig. 2 shows the expanded first derivative spectra of the 50, 200 and 250 mg tablets, corresponding to 8.32, 33.8 and 41.6% (w/w) of the API, respectively. By visual comparison of the spectra, Fig. 2 confirms that a desired NIR signal–API concentration relationship exists for this particular application.

3.2. Calculation of NIR signal intensity

Separation of API signals from the placebo can be achieved by using suitable mathematical pretreatment (e.g., first or second derivative). However, calculation of the signal intensity should be decided on a case-to-case basis. For easier explanation, a function ΔIntensity $(=(I_H - I_L) \times 10^4$, where I_H is the signal intensity at a high point and I_L at a low point) is arbitrarily defined. For the 200 and 250 mg CR tablets, I_L is the trough at 2110 nm (Fig. 2 in the 2100–2120 nm range), which represents the starting point of the API signals, while I_H is the maximum at 2140 nm. For the 50 mg strength tablet, due to the overwhelmingly strong excipient band, the starting point I_L is shifted to 2128 nm. The intensity difference between 2128 and 2140 nm was used for the CU calculation.

NIR signals of the API from the CR tablets with three different strengths were normalized against API concentration and presented in Table 1. The normalized responses were calculated using the following equation:

$$\text{Normalized NIR response} = \frac{\Delta\text{Intensity}}{\%\text{w/w API concentration}}$$

This value may be used as an additional assurance with regard to "purity" of the NIR signal. An excessively high value may represent the presence of interference from other components. The normalized NIR response from the 50 mg tablet sample was very close to the value of the drug substance sample whereas the 200 and 250 mg tablets show a significant deviation (up to about 40%). However, this deviation does not prevent application of the SQ-NIR method. Further discussion is given in following sections.

3.3. Effects of process parameters on NIR determination

To ensure reliability and correct interpretation of the results, boundaries of the SQ-NIR method need to be explored and defined. Various factors such as compression force and particle size distribution of the API may have specific implications.

3.3.1. Compression force

Fig. 3a demonstrates the effect of compression force on diffuse reflectance NIR absorption of the dose proportional IR tablets. The three clusters of raw spectra from top to bottom were obtained by scanning the IR tablets made with a compression force of 10.0, 6.3 and 4.5 kN. A brief look gives the impression that the spectra have a baseline shift due to the compression force difference. The NIR spectra for tablets compressed with higher compression force would shift higher than those compressed with lower compression force (because of differences in tablet hardness). However, the expanded first derivative spectra of the same samples (Fig. 3b) show that the differences in

Table 1
Normalized NIR responses

Sample	API (%w/w)	NIR signal, intensity ($\times 10^4$)	NIR response, intensity/(API, %w/w)
50 mg tablet	8.32	2.46	29.6
200 mg tablet	33.8	6.19	18.3
250 mg tablet	41.6	7.32	17.6
Drug substance	100.0	29.0	29.0

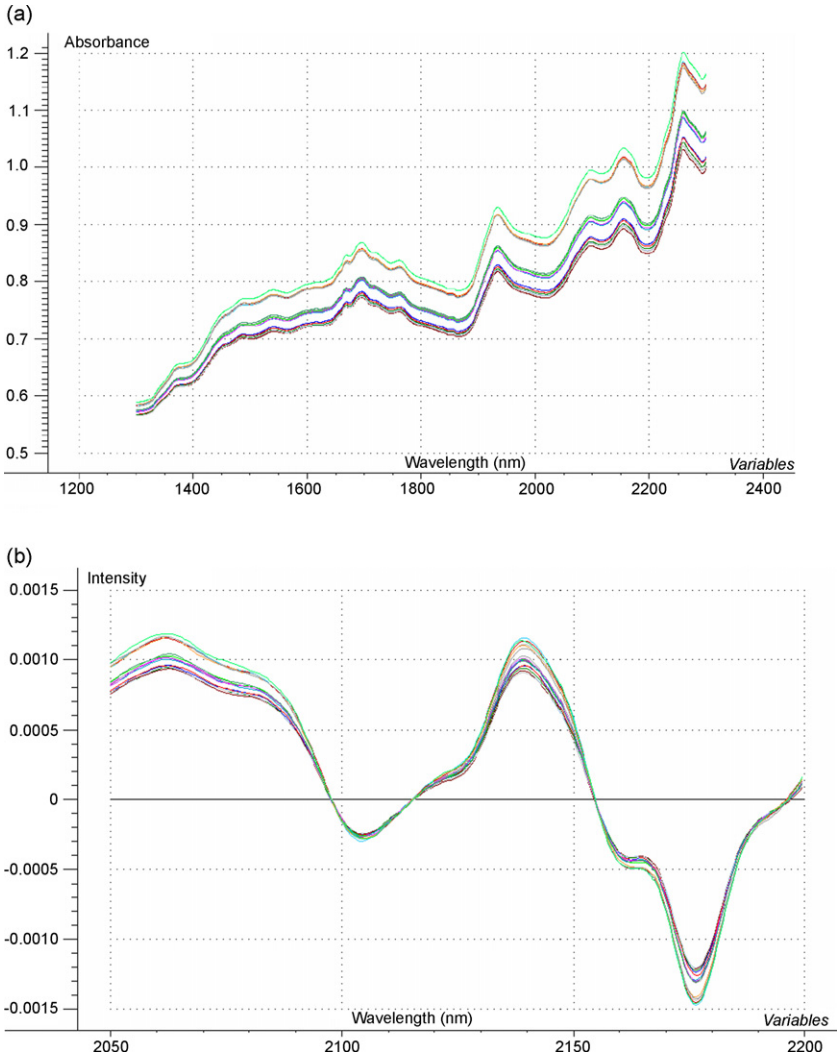

Fig. 3. Raw (a) and expanded first derivative (b) spectra of 75 mg IR tablets (46.6% API content) compressed at 10.0, 6.3 and 4.5 kN.

compression force not only have caused baseline shifts, but also have changed the ΔIntensity. Corresponding to the compression force of 10.0, 6.3 and 4.5 kN, the mean ΔIntensity values were 14.0 ($n = 30$), 12.7 ($n = 30$) and 11.8 ($n = 30$), respectively. These differences in ΔIntensity values cannot be eliminated by using other pretreatment methods (e.g., second derivative). Because the ΔIntensity values are directly used for CU (and BU) calculation, a significant fluctuation of compression force during tablet manufacturing will cause a variation in tablet hardness. This in turn will cause a higher %R.S.D. by the SQ-NIR method compared with the conventional HPLC or UV–VIS method. However, the ability of the SQ-NIR method to detect tablet hardness variations can be an advantage as long as the results are correctly interpreted.

3.3.2. API particle size

The impact of API particle size on ΔIntensity was also investigated. Two batches of the 50 mg CR tablets were prepared with drug substance lots that have significantly different particle size distribution ($D_{50} = 60$ μm versus 115 μm). Fig. 4 shows the raw and expanded first derivative spectra of the tablets. With an increase of D_{50} from 60 to 115 μm, a change in mean ΔIntensity from 1.98 ($n = 30$) to 2.51 ($n = 30$) was detected for the tablet samples. It is interesting to note that the first derivative (or second derivative, results are not shown) treatment could not eliminate the variations in NIR responses caused by the difference in particle size distribution. This observation implies that segregation of API particle alone would cause the SQ-NIR to detect higher %R.S.D. compared with conventional HPLC or UV–VIS method. Again, this can be an advantage for the NIR method as long as the results are correctly interpreted.

3.4. Interpretation of SQ-NIR results

As discussed in Section 3.3, CU results obtained by the SQ-NIR results are related not only to the amount of API (in the surface layer), but also to physical properties of the tablets. The variance measured is the sum of multiple terms:

$$\sigma^2_{(\text{SQ-NIR})} = \sigma^2_{(\text{real CU})} + \sigma^2_{(\text{non-linearity})} + \sigma^2_{(\text{compression force})} + \sigma^2_{(\text{particle size})} + \sigma^2_{(\text{other})}$$

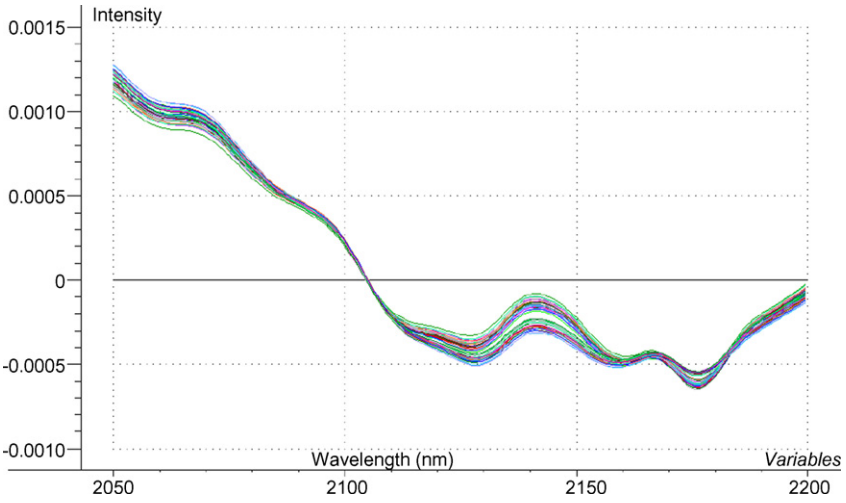

Fig. 4. Expanded first derivative spectra of 50 mg CR tablets with different API particle size.

The term $\sigma^2_{(\text{real CU})}$ is equivalent to the variance (content uniformity) measured by the reference method. The term $\sigma^2_{(\text{non-linearity})}$ takes into consideration of non-linear response of the NIR signals. Because CU is calculated based on the ratio of signal variations to the mean of absolute signal intensities, reduced signal intensity will affect the final CU results. The terms related to compression force and particle size distribution have been discussed. For tablets of good quality, the $\sigma^2_{(\text{real CU})}$ term is dominant. Therefore, the reference method and SQ-NIR method should generate similar CU results. For tablets of bad quality, the other terms may become dominant compared with the $\sigma^2_{(\text{real CU})}$ term. In those cases the CU results by SQ-NIR may show higher %R.S.D. compared with a reference method. When powder blend samples are analyzed, the variance caused by sampling may make a significant contribution to the overall $\sigma^2_{(\text{SQ-NIR})}$.

3.5. CU determination of intact tablets

Based on the above discussions, the SQ-NIR method can be used for CU analysis of intact tablets. Numerous batches of IR tablets with different formulations/strengths were prepared

Table 2
CU results for IR tablets by SQ-NIR and a reference method

Strength (mg)	Compression force (kN)	CU by NIR (%R.S.D., $n = 10$)	CU by ref[a] (%R.S.D. $n = 10$)	Composite assay by ref[a] (%)
Homogeneous batches[b]				
40	3.0	1.3	1.2	100
40	4.0	1.5	1.0	101
40	8.0	1.2	2.1	99
40	10.5	1.2	1.7	100
40	3.5	1.9	1.4	100
40	6.9	3.3	2.4	99
50	5.1	1.7	0.9	100
50	6.8	1.8	0.8	100
50	12.0	1.1	1.5	103
75	4.5	0.8	0.4	100
75	6.3	0.8	0.2	100
75	10.0	0.8	0.5	99
100	4.5	0.5	0.5	100
100	6.4	1.0	0.7	100
100	11.0	0.7	0.7	101
Inhomogeneous batches[b]				
40	3.5	14.3	2.4	86
40	6.9	6.1	3.4	87
40	2.7	9.4	1.5	87
40	11.7	10.4	2.4	86
50	2.0	16.8	1.8	87
50	6.5	16.9	14.4	96
50	10.5	7.6	3.0	89

[a] ref: reference method by UV–VIS.
[b] The same manufacturing process was used for these batches as specified in Section 2.3.

Table 3
Blend uniformity vs. content uniformity results for 50 mg CR tablets by SQ-NIR

Batch number	BU and CU in %R.S.D. ($n = 10$)	
	BU of thief samples	CU of tablets
1	3.5	2.5
2	3.1	2.6
3	1.5	2.4
4	3.3	1.8
5	1.8	2.0
6	4.3	2.9
7	2.9	2.2

during formulation development. These batches were analyzed by the SQ-NIR without calibration models and a UV–VIS reference method. The CU results by both methods and the composite assay results (mean of CU results by reference method, $n = 10$) are presented in Table 2. For most of the batches, the composite assay results are within 95–105% and the CU results have <5% R.S.D. by the reference method. These are considered the "good" or homogenous batches. Low composite assay and/or high %R.S.D. CU results were observed by the reference method for several "bad" or inhomogeneous batches. For the good batches the CU results by SQ-NIR are comparable with those obtained by the reference method. It is interesting to note that for most of the bad batches, the reference method gave perfectly good CU results even though the composite assay results were very low. However, the SQ-NIR method show consistently high %R.S.D. for the bad batches. For some of the bad batches, the cause was easily identified simply by visually inspecting the raw NIR spectra because they showed a signature pattern as in Fig. 3. Other bad batches were due to problems related to the wet granulation process. Discussions for those issues are out of the scope of this article.

3.6. BU determination

BU becomes more important for formulations that have low API content. A seven-batch study was conducted for the 50 mg CR formulation (8.32% API content). Mechanical thief samples were collected at the end of powder blending and analyzed using the SQ-NIR method. Later tablets were compressed and CU analysis was conducted for each batch using the same NIR method. Table 3 compares the BU and CU results from these batches. It is interesting to note that the CU results have relatively narrow spread compared with the BU results. For example, the BU result for batch 6 was 4.27%, which is much higher than the corresponding CU result. It is well understood in the industry that sampling through a mechanical thief sometimes introduces additional error and the so obtained BU results may be misleading.

4. Conclusions

BU and CU analyses are unique to the pharmaceutical industry. A SQ-NIR method is well suited for these tests because of its efficiency and information-rich nature. Compared with the conventional approach of quantitative analysis by NIR, the SQ-NIR approach eliminates the need for calibration models. This will greatly simplify implementation of the method in early and late stage pharmaceutical development alike. The method may be adopted as an independent test method. It may also be used along with the traditional HPLC or UV–VIS method. Of course, a composite assay method is still needed for potency and impurity determination. Compared with the traditional HPLC or UV–VIS method, it is important to keep in mind that the CU results by a SQ-NIR method may contain additional information related to the products therefore should be interpreted accordingly. In addition, a SQ-NIR method may not work for APIs that do not have the suitable functional group(s) (e.g., RNH_2) or are present at very low levels.

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