Non-destructive quality control of pharmaceutical tablets by near infrared reflectance spectroscopy

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Introduction

In today's highly competitive business world, it is vital for companies to produce consistently high-quality products at the lowest possible cost. This can be achieved if the production process is monitored and controlled by quick and reliable methods of quality assurance/quality control (QA/QC). The issue of QA/QC is especially important in the pharmaceutical industry, as impurities or wrong specifications for active ingredients in finished products can cause dangerous, and sometimes, fatal consequences to consumers. Pharmaceutical companies often deal with a large number of raw materials and, without careful monitoring, confusion between two similar substances can easily happen. In addition, the incoming raw materials have to be tested to meet certain specifications before they can be used in the production process. Therefore, manufacturing high-quality pharmaceutical products requires at least two steps of quality analysis: identification of the incoming raw materials and quantitative analysis of active ingredients in finished products. In both of these important steps, near infrared (NIR) spectroscopy, used in combination with fiber optic probes, has been proven to be an easy-to-use and powerful tool.

By using fiber optic probes, NIR spectra can be collected directly from both solids and liquids without any sample preparation. The measurements can often be made non-invasively and non-destructively through glass vials or thin polymer films. High quality spectra can generally be collected in a few seconds because of the brighter source and more sensitive detectors used in the NIR region versus the mid-infrared region. Moreover, in contrast to time-consuming wet chemical analysis, the NIR method produces no wastes, causes no pollution and requires no chemical reagents. The NIR analysis accuracy is generally much better than that of wet chemical analysis. All of these above mentioned factors make NIR spectroscopy a quick, reliable and inexpensive analytical method for QA/QC work in the pharmaceutical industry.

Identification

In order to identify an incoming raw material, the spectra of the delivered raw materials need to be compared with standard spectra in a software library. The standard spectrum of a certain substance is the average spectrum of multiple spectra collected from several acceptable batches of this substance. This standard spectrum is often referred to as the "library spectrum", and these multiple individual spectra are often called "reference spectra". If a sample is identical to a

substance whose reference spectrum is stored in the library, then, the spectrum collected from this sample will be very similar to its corresponding library spectrum. This similarity is expressed in terms of the so called Euclidean distance D_{sample}:

$$D_{sample} = \sqrt{\sum_{i=1}^{k} [A_{sample}(k) - A_{lib}(k)]^2} \quad (1)$$

$A_{sample}(k)$: absorbance of the sample spectrum at wavelength k.
$A_{lib}(k)$: absorbance of the library spectrum at wavelength k.

Equation 1 indicates that the more similar a spectrum is to the library spectrum, the smaller the Euclidean distance D_{sample}. If two spectra are absolutely identical [$A_{sample}(k) = A_{lib}(k)$ for every k] then D_{sample} will be zero.

Setting up a library for substance identification typically requires two steps: first, a couple of measurements on several acceptable batches have to be performed. The average spectrum is then calculated and stored as a standard spectrum in the library. The spectra of all future samples are compared with this average spectrum. Second, a threshold D_T, which defines the tolerance of the identification model, is calculated. The optimum algorithm for such a threshold calculation depends on the system under examination. For example, if the value of D_T is too high, the model will accept incorrectly the sample spectra which shows only small similarity to the corresponding library spectrum. Consequently, these materials will be identified as identical substances, though they are not. On the other hand, if D_T is set too low, the model will not be flexible enough and a small natural deviation in the sample spectrum may cause the model to reject the sample which otherwise should be accepted. In this paper, an empirical algorithm is used for the threshold calculation. This method has been proven in the past, to be able to create stable and reliable identification models. In this algorithm, the threshold D_T for a particular substance is calculated as follows:

$$D_T = D_{sample,max} + \frac{S_0}{4} \quad (2)$$

$D_{sample,\ max}$ is the maximum Euclidean distance of all n reference spectra collected to set up the library and S_0 is the standard deviation of the Euclidean distances of all n reference spectra:

$$S_0 = \sqrt{\frac{\sum_{i=1}^{n} D_{sample,i}^2}{n-1}} \quad (3)$$

There are three possible cases during the identification process. First, if the Euclidean distance of the spectrum of a query is below the threshold of only one substance, say A, in the library, then the query can be uniquely identified as substance A,

D_{sample} (query) < D_T (A)
D_{sample} (query) > D_T (all other substances)
$\Rightarrow$ query is identical to A.

Second, the Euclidean distance of the spectrum of a query is below the thresholds of two or more other substances in the library, say A and B. Then the query cannot be uniquely identified as either A or B. In this case, the query can be “confused ” between A and B.

D_{sample} (query) < D_T *(A)*, D_T *(B)*
D_{sample} (query) > D_T (all other substances) $\Rightarrow$ query can be either *A* or *B*.

Third, a query will be found to be not identical to substance *A* if the Euclidean distance of the query spectrum is larger than the threshold of substance *A* in the library

D_{sample} (query) > D_T *(A)* $\Rightarrow$ query is not identical to *A*.

Quantitative analysis

Unfortunately, the absorption bands in the NIR region are generally broad and seriously overlap. Conventional univariate calibration techniques, using only one wavelength per component for evaluations, do not work in cases of overlapping bands. The lack of isolated bands was one of the main reasons that NIR spectroscopy has been largely ignored by analytical laboratories until recently. The development of more sophisticated statistical tools, such as multivariate analysis, has revitalized the broad application of NIR spectroscopy in many industries. One of the most widely used multivariate methods is partial least-squares (PLS) regression.

The theory of PLS regression and its application in vis/NIR/IR spectroscopy has been reported by several authors.[1–4] This technique usually requires two steps to set up a calibration model: calibration and prediction. In the calibration step, the spectral data (absorbance values at different wavenumbers) and the known concentration values for a set of calibration samples are used to calculate so-called "latent variables" of the spectral response matrix and the concentrations of these components. These latent variables or factors (linear combinations of the original variables) account for the variance in both the spectral and concentration matrix. Hence, they are not directly measurable, but summarize the most important information in both data sets. One of the main advantages of PLS regression over other multivariate calibration techniques is that it simultaneously estimates underlying factors in both the spectral response matrix and the concentration matrix during the modeling process. Therefore, if no noise is present in the data matrices, the variation in the factors of spectral data will be directly connected to the variations in the factors of the concentration data matrix. In addition, PLS can be used to remove spectral noise in the data matrix by excluding additional (higher) factors in the model, that mainly describe noise.[6]

In the prediction step, the optimum number of factors determined from the calibration set are used to calculate the analyte concentrations of unknown samples by correlating these latent variables with the spectral data of the unknowns. Since multivariate analysis uses more spectral information, it generally provides a higher accuracy in concentration prediction than univariate analysis.

Experimental

All NIR spectra in this work were collected from 12,000 to 4000 cm^{-1} (833–2500 nm) with a fiber optic probe connected to a FT-NIR spectrometer (Bruker IFS 28/N). The probe was used to collect diffuse reflectance spectra from tablets of acetyl salicylic acid (ASA) and ten powder samples commonly used in the pharmaceutical industry. A total of 16 scans were collected and averaged for both background and sample measurements. A new background was taken with a spectralon standard for each series of sample measurements. For substance identification, five spectra of each sample were collected by immersing the probe directly into the powder. The spectral resolution was set at 8 cm^{-1}. The tablet measurements for quantitative analysis were carried out in two different ways. First, the tablets were measured by pressing the fiber optic probe directly on the surface of the tablets. Here, 8 cm^{-1} resolution was used. Second, tablets were

measured non-invasively by pressing the probe on the blister pack. In this case, the spectra were collected with resolutions of 2, 4, 8, 16 and 32 cm^{-1}.

All substances used in this study were provided by a pharmaceutical company in Germany. The ASA tablets were produced by blending a weighed amount of acetyl salicylic acid with two types of starches (filler materials). After the NIR spectra were collected, the concentrations of these three components were determined by HPLC measurements. The concentration values of each tablet, calculated from the weights of the starting materials, agreed very well with the corresponding HPLC results.

Results and discussion

Identification

Ten commonly used components of pharmaceutical products were used to set up a library for substance identification. For each sample, the Euclidean distance D_{sample} and the threshold D_T was calculated according to Equation 1 and 2. The result is shown in Table 1, using ASA as an example. The Euclidean distance for the measured sample spectrum D_{sample}(ASA) is 0.0109483, while the corresponding threshold for ASA D_T(ASA) is 0.04674480. Obviously in this case, the Euclidean distance of ASA is smaller than the threshold of ASA in the library:

$$D_{sample}\,(\mathrm{ASA}) < \mathrm{D_T}\,(\mathrm{ASA}).$$

In addition, by examining Table 1, it is easy to find that the Euclidean distance of ASA is larger than the thresholds of all other substances in the library:

$$D_{sample}(\mathrm{ASA}) > \mathrm{D_T}\ (\text{every other substance}).$$

Table 1. Euclidean distances of acetyl salicylic acid and the thresholds for every substance stored in the library (spectral range: 12,000–4000 cm^{-1}; data preprocessing: vector normalized; spectral resolution: 8 cm^{-1}).

No.	Euclidean distance D_{sample} (ASA)	Threshold D_T (Substance)	Substance
1	*0.0109483*	*0.04674480*	*Acetyl salicylic acid (ASA)*
2	0.187451	0.00888951	Salicylic acid
3	0.259888	0.02077080	Salicylamide
4	0.446525	0.01406960	Collidon 25
5	0.455598	0.00490291	Collidon 30
6	0.472364	0.00663132	Corn starch, soluble
7	0.499913	0.02075560	Corn starch
8	0.506830	0.00584698	Avicel PH 101
9	0.508482	0.02169890	Avicel PH 102
10	0.510495	0.01377080	Potato starch

With these two conditions satisfied, it can be concluded that the sample ASA can be uniquely identified as acetyl salicylic acid and will not be confused with any other substance in the library.

Moreover, the value of D_{sample} of every compound in this example is not only smaller than the D_T value of its corresponding substance in the library but also greater than the thresholds of all other substances. So, each of the ten substances can be correctly identified without risk of confusion. The above example demonstrates that this empirical model can be used as an identity check for incoming pharmaceutical raw materials.

Quantitative analysis

Calibration spectra were collected for a series of tablets of acetyl salicylic acid. The concentration range of ASA was between 85 to 90%. In addition to ASA, the tablets consisted of two types of starch in the range of 0–10%, respectively.

The absorption bands of most organic compounds in the NIR region are due to C–H, N–H and O–H overtones and combination vibrations. All of these appear in a very narrow wavelength range.[7] Therefore, if the concentration of ASA in a multi-component system needs to be determined, multivariate calibration techniques such as PLS regression appear to be the only way to correlate the overlapped spectral data with the concentrations of the components of interest.

To set up a PLS calibration model, a total of 44 spectra were collected from 7200 to 4500 cm^{-1} at 8 cm^{-1} resolution. The optimum rank for ASA is determined to be three by the cross-validation procedure. The true concentrations of the ASA are plotted against the predicted concentrations in Figure 1. It can bee seen that by employing a PLS calibration a good agreement between true and predicted concentration data was found. The root mean squared error of cross-validation, *RMSECV*, was 0.35% and the explained variance R^2 is 93.8%. This is a very satisfactory result, since the German government requires the concentrations of ASA in pain killer tablets to be within

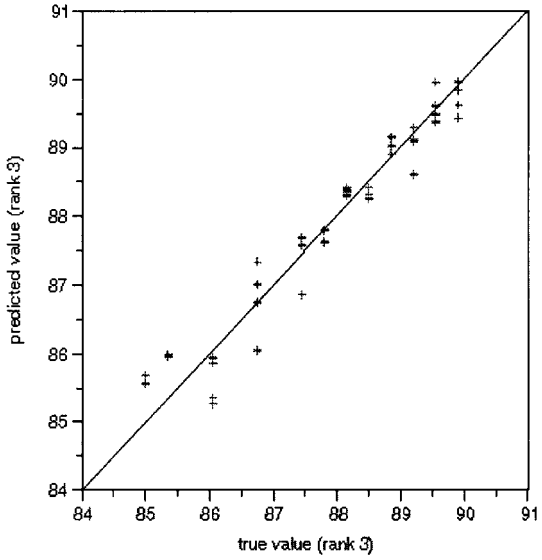

Figure 1. Direct measurement of ASA tablets. Comparison between true and predicted concentration data of acetyl salicylic acid (44 calibration spectra, 3 components, vector normalized spectra, spectral range: 7200–4500 cm^{-1}, resolution: 8 cm^{-1}).

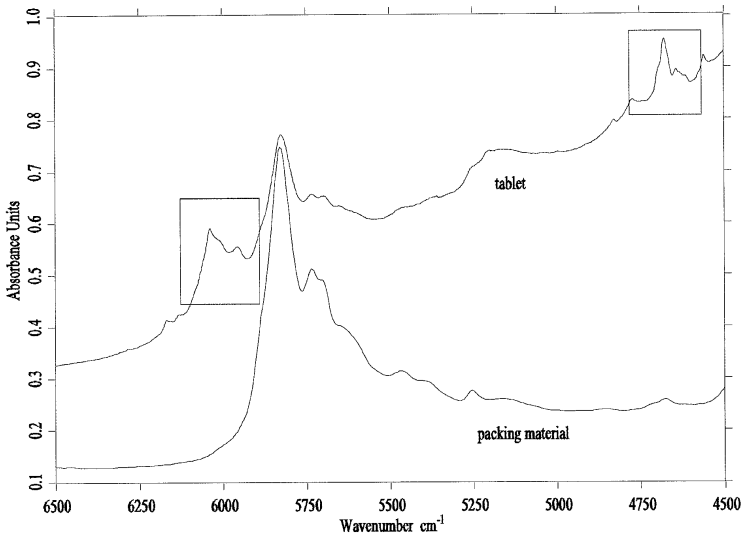

Figure 2. NIR spectra of acetyl salicylic acid measured through the blister pack and the polymer of the blister pack itself. The marked spectral regions show the wavelength range used for calibration.

±5%. Obviously, the error of prediction achieved by this PLS model is sufficient for non-destructive analysis of such tablets by NIR spectroscopy.

In addition to direct diffuse reflectance measurements of ASA at the tablet surfaces, ASA was measured non-invasively through the polymer blister pack. The absorption bands of the polymer films dominate the resulting spectrum. Figure 2 shows a spectrum of blister packaging material plotted along with a spectrum of a tablet measured through the blister pack. Only two spectral regions show significant aborption bands associated with the tablet: the region from 6070 to 5900 cm^{-1} and the region from 4730 to 4580 cm^{-1}. Only these two regions were used as a spectral window to set up a calibration model. A total of 42 calibration spectra were collected at 2 cm^{-1} resolution.

The result of the cross-validation for the corresponding three-factor PLS model is shown in Figure 3. Again, an accurate result is achieved with a mean prediction error of less than 0.5% (*RMSECV* = 0.46%, R^2 = 91.30%). So, the conclusion can be made that it is generally possible to determine the amount of ASA in tablets by NIR spectroscopy and multivariate techniques.

This result might seem surprising, since poor prediction results are generally expected from a calibration model based on a narrow spectral region with overlapping bands from interfering substances. On the contrary, the presented PLS model shows satisfactory prediction results. The corresponding values of *RMSECV* and R^2 of the measurement made through the blister pack are comparable to those from the direct measurement of the tablets. In the following, it will be demonstrated that these good prediction results, in case of measuring through the packaging material, is mainly due to the higher spectral resolution used in the study.

Influence of resolution on the accuracy of a PLS model

If the diffuse reflectance data were collected with a resolution less than 8 cm^{-1}, much spectral information is lost. This is illustrated in Figure 4 with one of the frequency windows

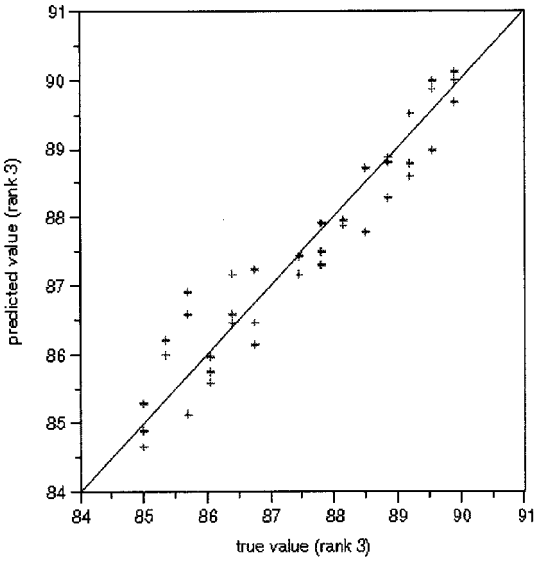

Figure 3. Measurement of ASA tablets through a blister pack. Comparison between true and predicted concentration data of acetyl salicylic acid (42 calibration spectra, 3 components, vector normalized spectra, spectral range: 6070–5900 cm^{-1} and 4730–4580 cm^{-1}, resolution: 2 cm^{-1}).

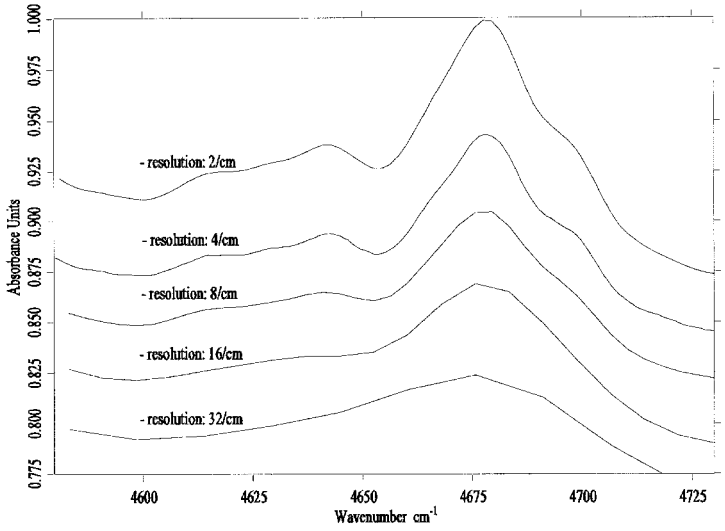

Figure 4. The influence of spectral resolution on the shape of an absorbance band of acetyl salicylic acid in the near infrared region (measurement through the polymer film of a blister pack).

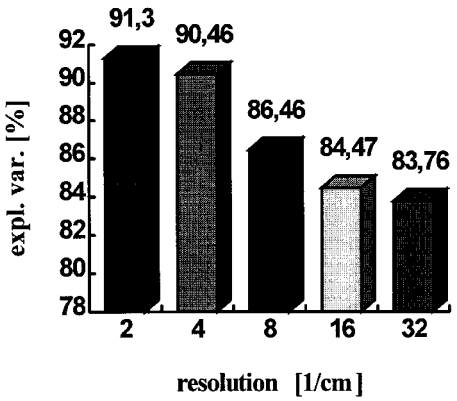

Figure 5. The influence of spectral resolution on the variance explained by the PLS model.

(4730– 4580 cm^{-1}). It can be seen that the true characteristic of the absorption band is clearly represented if high spectral resolutions are used. On the contrary, as the spectral resolution decreases, the spectral bands become flatter and more featureless, hence, contain less useful spectral information. Therefore, an accurate calibration model with good prediction results can only be found with higher resolutions.

The influence of the spectral resolution on the accuracy of a PLS model is illustrated in Figure 5. Here, the explained variance R^2 is plotted versus the resolutions for the tablet measurements through the packaging material. The values of R^2 clearly decrease as the resolution decreases from 2 to 8 cm^{-1} or less. This means a substantial decrease in the accuracy of the corresponding analysis results. The *RMSECV* increases from 0.460 to 0.593% if the resolution is set from 2 cm^{-1} to 32 cm^{-1}. Therefore, in this example, a resolution of 2 or 4 cm^{-1} is required to create a satisfactory calibration model with errors of prediction less than 0.5%.

Conclusions

NIR spectroscopy, when used in combination with fiber optic probes, provides a quick, easy-to-use and reliable tool for quality assurance and quality control in the pharmaceutical industry. Multivariate calibration methods, such as PLS regression, can be successfully applied to quantitative determinations of active ingredients such as acetyl salicylic acid in tablets. Moreover, in difficult situations, i.e. strongly overlapping bands from interfering components, or if only a very narrow spectral region is available for calibration, more accurate prediction results can be achieved by increasing the spectral resolution to 2 cm^{-1}.

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