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Application of standardisation methods to correct the spectral differences induced by a fibre optic probe used for the near-infrared analysis of pharmaceutical tablets[1]

E. Bouveresse [a], C. Casolino [a,*], C. de la Pezuela [b]

[a] *Dipartimento di Analisi e Tecnologie Farmaceutiche ed Alimentari, Via Brigata Salerno (Ponte), I-16147 Genova, Italy*
[b] *Departamento de Química, Unidad de Química Analítica, Universidad Autónoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain*

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Abstract

Near infrared spectroscopy has become very popular in the pharmaceutical industry because of many important practical advantages. With the help of powerful chemometric techniques, multivariate calibration models are developed, relating near-infrared spectra to the values to be modelled. However, because of small instrumental differences between near-infrared spectrometers, a calibration model can only be used with spectra collected on the same instrument, which represents a serious limitation for the use of near-infrared spectroscopy in the pharmaceutical industry. To deal with this important problem, a certain number of different standardisation approaches were proposed in the literature. In this article, an application of instrument standardisation methods is presented, where two different data measurement modules (internal measurement cell and external fibre optic module) of a near-infrared spectrometer must be standardised for the quantitative determination of an active compound in pharmaceutical tablets. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Near-infrared spectroscopy; Instrument standardisation; Calibration transfer; Multivariate calibration; Fibre optic module

1. Introduction

1.1. Near-infrared spectroscopy in the pharmaceutical industry

Near-infrared (NIR) spectroscopy has become one of the most powerful techniques in analytical chemistry [1–4] and particularly in the pharmaceutical industry [5], because of the following important advantages. (a) The energy of NIR

* Corresponding author. Fax: +39 10 3532684; e-mail: chiara@anchem.unige.it

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radiations is small enough to allow a non-destructive analysis of samples. (b) However, contrary to mid-IR radiations, the energy of NIR radiations is high enough to allow longer path lengths through the sample without the radiation being completely absorbed. Therefore, NIR spectroscopy enables the analysis of a wider variety of samples, including for instance strongly absorbing samples and opaque solid materials. (c) Moreover, NIR radiations also allow the use of long fibre optics, which can be very useful, e.g. for the on-line analysis of pharmaceutical blends [6,7]. (d) Another advantage of NIR spectroscopy is that it requires little or no sample preparation and it therefore enables easy and fast data collection. (e) NIR spectroscopy is a non-destructive method, which represents a considerable advantage for applications, where the analysed samples must not be altered (e.g. biological and medical applications [8]). Moreover, this enables NIR spectroscopy to be used for the on-line monitoring of industrial processes [6,7]. (f) NIR spectroscopy enables the determination of several physico–chemical properties and/or concentrations of chemical compounds from a single spectrum. This is particularly important for quality control applications [9], where a lot of different properties and/or concentrations must be determined for a high number of routine samples. (g) At last, an additional practical advantage of the NIR spectroscopy is that there is a number of materials, such as glass or translucid plastic, which almost do not interact with the NIR light. If samples are packed in such materials, NIR light can be directly sent through the packing material, in order to analyse the samples. Therefore, NIR spectroscopy can be used for instance, to analyse tablets through the plastic blister [10,11].

1.2. Development of near-infrared calibration models

NIR spectroscopy can be used as primary method if artificial calibration samples with the same composition and structure as the samples to be analysed can be realised and measured on the NIR spectrometer. This can certainly be applied for very simple mixtures: however, most of the industrial products are complex matrices, which cannot be simply reconstructed. Therefore, NIR spectroscopy is mostly used as a secondary method. Because the NIR spectrum of a sample does not contain the information enabling the direct determination of a studied parameter, it is necessary to develop NIR calibration models [12].

The development of NIR calibration models consists of collecting the spectra of calibration samples on an NIR instrument, and analysing these calibration samples by the reference method to obtain the corresponding reference value of the studied parameter (e.g. concentration of an active compound in a tablet) for each calibration sample. The development of a calibration model involves determining the mathematical relationship relating the NIR spectra and the corresponding reference values of the studied parameter. Since NIR spectra are a complex combination of overlapping absorption bands, powerful chemometric methods must be used to extract the useful information from these complex NIR spectra [13]. The computed calibration coefficients are used to predict the values of the studied parameter for new unknown samples, which are therefore not analysed by the usually time-consuming reference method. After having been computed, the calibration model must be validated with independent test samples. Those test samples are measured by both NIR and reference methods to check that both primary and secondary methods are in good statistical agreement.

When the calibration model has been validated, it can be used to predict the studied parameters for new samples. However, good predictions can only be obtained if the NIR spectra of the new samples are within the experimental domain covered by the calibration samples. Since extrapolation of the calibration model outside the calibration experimental domain leads to erroneous predictions, it is usually more convenient to develop calibration models on very large data bases, in order to cover all possible sources of variations. In practice, calibration data bases can sometimes involve several hundreds of calibration samples collected over very long periods of time. Therefore, the development of such calibration models requires considerable effort, important costs and long time delays.

1.3. Instrument standardisation

There are a number of situations in which a calibration model can become inapplicable. Two different sources of changes can be distinguished.

The first one can occur when the instrumental response is fluctuating in time (e.g. continuous drift due to instrument ageing, or sudden shift due to instrumental repair) and/or when the measurement conditions are significantly modified (e.g. temperature). If such fluctuations occur, predictions computed with calibration models developed at a certain time and NIR spectra collected after a certain period of time will be erroneous. It is therefore essential to check the stability of the instrument, on which calibration models were developed. For this purpose, a strategy for the maintenance of NIR calibration models over time has been developed and successfully applied to assess the stability of a NIR instrument during 15 months [14]. In this strategy, fluctuations are detected by means of validation tests [14,15], and two different approaches were developed to correct these instrumental changes, the first one based on simulation of fluctuations [16] for simple instrumental changes (e.g. constant offset in absorbancies), the second one based on standardisation algorithms [17,18] for more complex instrumental changes.

The second source can occur when a calibration model developed on a first NIR instrument (usually referred to as 'primary' or 'master' instrument) has to be used with spectra collected on a second NIR instrument (usually referred to as 'secondary', 'slave', 'server' or 'host' instrument). Each NIR instrument has its own instrumental response, this response being different from the one of another instruments, even if both instruments are identical (same characteristics, same manufacturer). Because of these different instrumental responses, predictions computed with calibration models developed on a master instrument and NIR spectra collected on a slave instrument will be erroneous. However, in many industrial applications and for quality control nets [19], NIR users would like to avoid developing and maintaining calibration models on each NIR instrument separately, which would imply considerable work and time. To avoid this fastidious work, standardisation methods [17,18] have been proposed in order to correct these instrumental differences between NIR spectrometers. The calibration development and maintenance is then limited to a single master NIR instrument, the other slave instruments being used for the collection of NIR data only. In the literature, a number of standardisation methods have been proposed [20–25] to deal with this important problem. As for the maintenance of calibration models [14], an overall strategy to select the most suitable standardisation method was developed in order to help NIR users to select the most suitable standardisation approach [26].

1.4. Standardisation of two measurement modules

In this article, standardisation approaches are applied to correct differences due to the use of different measurement modules (internal measurement cell and external fibre optic module) of a NIR monochromator instrument used for the quantitative determination of an active compound in pharmaceutical tablets. Indeed, calibration models are developed on NIR instruments by measuring calibration samples usually with the internal measurement cell. However, for particular applications such as on-line analysis [6,7] or tablet identification [10,11], the NIR spectra are often collected via a fibre optic module which enables to send the light from the spectrometer to the product and vice versa. Therefore, it is essential to correct differences due to the different measurement modules, in order to keep satisfactory predictions whatever the measurement module used for data collection.

2. Theory

2.1. Selection of suitable standardisation samples

The choice of the standardisation samples is a crucial step to obtain reliable standardisation parameters. Since instrumental differences are estimated only with standardisation samples measured on both instruments, the standardisation

samples must cover the experimental domain as well as possible, in order to determine standardisation parameters reliable for the whole experimental domain. However, only a reduced number of standardisation samples has to be used to determine the standardisation parameters, in order to minimise the work to be performed.

To select suitable standardisation samples, a good compromise has to be made between representativity and stability. The best approach to dispose from representative standardisation samples is to select a subset among the samples used for calibration. An algorithm able to select a few samples well spread over the experimental domain must then be used. From a previous study [27], it turned out that the most suitable algorithm for the selection of a representative standardisation subset was the algorithm proposed by Kennard and Stone [28]. After having been selected among the calibration data base, the standardisation samples are remeasured on the slave instrument to estimate the spectral differences. However, it should be pointed out that the standardisation samples must be perfectly stable between the moment at which they are measured on the master instrument and the moment at which they are measured on the slave instrument. If the analysed samples are not stable, more stable standardisation samples (e.g. generic standards) or pure chemicals can be used [22,29] (a pure chemical is not necessary stable over time, but substituting this pure chemical by the same newly produced pure chemical at regular time intervals allows to dispose from the same sample for standardisation purposes).

2.2. Selection of a suitable standardisation method

To choose the most appropriate method for a particular standardisation problem, a strategy was proposed [26] enabling the selection of the most simple standardisation approach. For calibration transfer, methods either based on transferring predicted y-values or NIR spectra can be used. $\mathbf{X}_1$ being the data matrix containing the NIR spectra of the standardisation samples collected with the internal measurement module, and $\mathbf{X}_2$ being the data matrix containing the NIR spectra of the standardisation samples collected with the fibre optic module, the approach based on transferring NIR spectra computes a set of standardisation paramaters F so that:

$$\mathbf{X}_1 = \mathbf{X}_2 \cdot F \tag{1}$$

F can be either computed by the Shenk–Westerhaus method based on a quadratic wavelength index correction followed by univariate linear regressions [21], by a modified version of this algorithm based on locally weighted regression models [22] (helpful with standardisation samples different from the one to be analysed), with the two-block partial least squares (PLS) method [24], with the piecewise direct standardisation (PDS) method [23], or with a standardisation method based on correcting spectral differences after wavelet transformation [25]. Each new spectrum x_2 collected with the fibre optic module can then be transferred and an estimation of the spectrum $x_{2\text{std}}$ which would have been obtained with the internal measurement cell is computed.

$$x_{2\text{std}} = x_2 \cdot F \tag{2}$$

The transferred spectrum can then be used with the b calibration coefficients to compute reliable predictions $\hat{y}_{\text{std}}$.

$$\hat{y}_{\text{std}} = x_{2\text{std}} \cdot b = x_2 \cdot F \cdot b \tag{3}$$

Concerning the standardisation based on predicted y-values [20], the calibration model is applied to the NIR spectra of the standardisation samples collected on both modules, yielding reliable predictions $\hat{Y}_1$ for the spectra $\mathbf{X}_1$ collected with the internal measurement cell, and erroneous predictions $\hat{Y}_2$ for the spectra $\mathbf{X}_2$ collected with the fibre optic module. The $\hat{Y}_2$ are corrected by univariate linear regression so that

$$\hat{Y}_1 = \text{slope} \cdot \hat{Y}_2 + \text{bias} \tag{4}$$

For each new spectrum x_2 collected with the fibre optic module, standardised predictions can then be obtained by computing erroneous predictions and by making a slope/bias correction of these predictions.

$$\hat{y}_{\text{std}} = \text{slope} \cdot \hat{y} + \text{bias} = \text{slope} \cdot x_2 \cdot b + \text{bias} \tag{5}$$

For data sets with simple differences, this strategy can yield very good results, but for more complex differences, this simple correction fails. To determine whether the slope/bias correction method can yield good results, a method based on a statistical *F*-test was proposed and successfully applied to different standardisation problems [30,31].

2.3. Validation of the standardisation step

To validate the standardisation step, it is necessary to test whether the determined standardisation coefficients indeed yield satisfactory results, when new samples are measured on the standardised slave instrument. Therefore, a procedure similar to the validation tests [15] is applied. A few new samples are analysed by the reference method to obtain reference *y*-values, the NIR spectra of those new samples are transferred from the slave to the master instrument with the determined standardisation coefficients, and the calibration model is applied to the transferred spectra, yielding predicted *y*-values, and the standard error of prediction (SEP) obtained for these samples is computed and compared to the expected SEP of the calibration model.

3. Experimental

3.1. NIR data set

The pharmaceutical data set used contains Mentis® tablets, a commercially available product from Laboratorios Menarini S.A. containing pirisudanol dimaleate as active compound. Since this data set has already been described in detail in the literature [32], only the information necessary to a good understanding of the standardisation problem will be given. A data set of 28 Mentis® tablets was measured on a NIRS 6500 instrument (Perstorp Analytical, Silver Spring, MD) with both a spinning sample module and an AP6641 ANO4P fibre optic module. The spinning cell is a rotatory conventional cuvette, and the collected NIR spectra are the average of a fixed number of scans taken while the cuvette holder

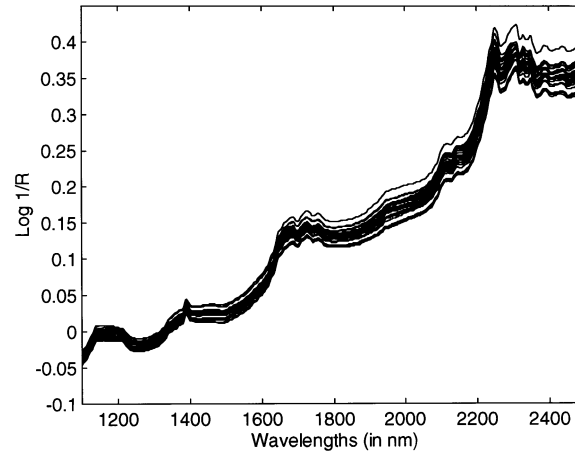

Fig. 1. NIR spectra of the Mentis® tablets collected with the spinning sample cell.

spins (minimisation of sample heterogeneity and light scattering due to differences of particule size). For both spinning and fibre optic modules, the NIR spectra were recorded in the range 1100–2500 nm with a 4 nm step (see Figs. 1 and 2), the samples were scanned in triplicates, and the three NIR spectra obtained were then averaged.

3.2. Reference method

The concentration of pirisudanol dimaleate in the tablets were determined by a reference method

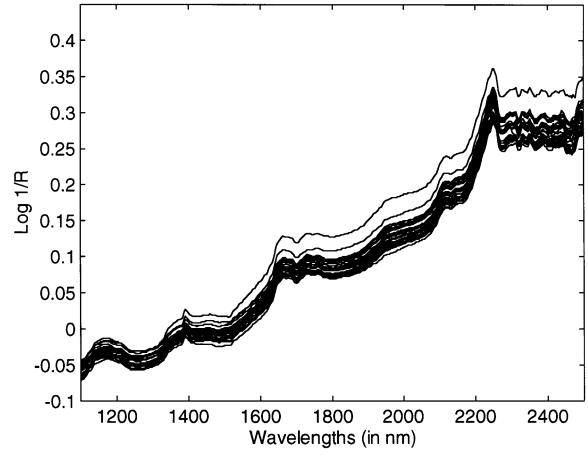

Fig. 2. NIR spectra of the Mentis® tablets collected with the fibre optic module.

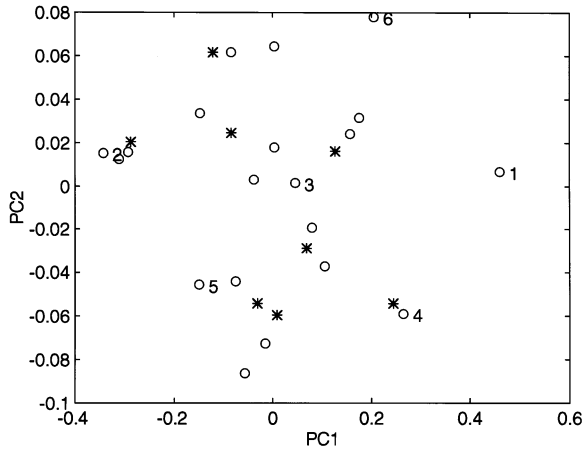

Fig. 3. PC1–PC2 score plot of the data set obtained with the spectra collected with the spinning sample cell. The samples belonging to the test set are represented with asterisks, the samples belonging to the calibration set are represented by circles, and the six calibration samples selected in the standardisation subset are indicated with numbers.

[32] based on sample grinding and dissolution, ultrasonic pretreatment of the solution, filtration, and ultra-violet spectrometric analysis of the obtained solution.

3.3. Software

The data sets were analysed with programs developed in the Matlab (The Mathworks, Natick, USA) environment.

4. Results and discussion

4.1. NIR calibration model

To predict the amount of pirisudanol in Mentis® tablets, a calibration was developed from the NIR spectra collected with the spinning sample module (considered as the 'master module'). The 28 samples were divided in two groups, namely 20 samples in the calibration set and eight samples in the test set. The separation was performed based on sorted reference y-values, one third of the samples being used as test samples. The samples with the lowest and highest y-values were put in the calibration set to avoid model extrapolation in the y-domain. The position of both calibration and test samples in the X-space were plotted in Fig. 3 to check that the test samples are well spread within the domain defined by the calibration samples. PLS regression was used to compute the calibration coefficients from the raw NIR data. The optimal complexity determined by leave-one-out cross-validation was found equal to 3. This model was validated by using the eight test samples, and a SEP of 6.6 mg g^{-1} was obtained.

4.2. Instrument standardisation

To see whether there are significant differences between the spectra delivered by both modules, the calibration model developed with spectral data collected with the spinning sample module were directly applied to the NIR spectra of the eight test samples measured with the fibre optic module (considered as the 'slave module'). The obtained SEP was about 53.2, which is eight times larger than the one obtained with NIR data collected with the spinning sample module. Standardisation of both modules was therefore necessary.

Regarding the selection of the most suitable standardisation samples, a representative subset of calibration samples was used in this case. Indeed, the studied samples can be considered as stable during the measurements on both spinning sample module and fibre optic module, since they are measured on the same instrument at the same place. A representative subset containing three to six standardisation samples was therefore selected with the Kennard and Stone [27,28] algorithm. The positions of these well-spread standardisation samples in the experimental domain are indicated on the score plot shown in Fig. 3, which presents the position of each sample in the space determined by the first two principal components (PC).

Concerning the choice of the most appropriate standardisation method, different points must be considered, such as the type of instrument considered, and the complexity of the spectral differences to be corrected. In the present case, the data are collected on the same NIR monochromator instrument with different measurement modules. Therefore, the NIR spectra collected with both

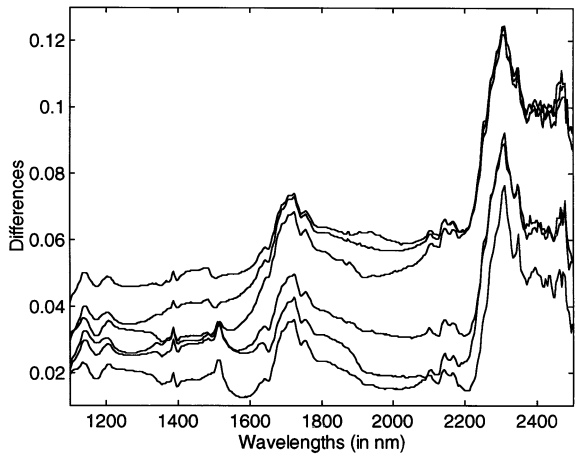

Fig. 4. Spectral differences between the NIR spectra of the six standardisation samples collected with the spinning sample cell and with the fibre optic module.

measurement modules are the same regularly sampled data sequence of spectral intensities at the same wavelengths. When plotting the spectral differences between both measurement modules (Fig. 4), it can be seen that the spectral differences are rather similar for all standardisation samples. Therefore, it is probable that a rather simple standardisation method can be used to correct these differences.

For rather simple spectral differences, two standardisation approaches are possible, the first one being the slope/bias correction of the predicted y-values (transfer based on the y-values), and the second one being the Shenk–Westerhaus algorithm (transfer based on the NIR spectra). If the user is only interested in having reliable predictions for future samples analysed with the fibre optic probe, the slope/bias correction of the predicted y-values can be applied. However, if the user would like to have the NIR spectra at his disposal for other purposes (such as e.g. updating a data base), a method based of transferring NIR spectra must be used.

To check whether the slope/bias correction method can be applied, the F-test already described in other references [30,31] was applied. Whatever the number of standardisation samples, the F-test always yields an experimental F-value smaller than the critical F-value, and this indicates that it is possible to successfully apply the slope/bias correction method. Table 1 indicates the different results obtained with both slope/bias correction method and Shenk–Westerhaus algorithm. In both cases, the SEP obtained with data collected on the fibre optic module after standardisation was brought to an acceptable value very close to the SEP obtained with data collected on the spinning sample module.

At last, it should be noted that the spectral data collected with the fibre optic probe were not very noisy because the length of the fibre optic device was very short (<1 m). Indeed, a calibration model was developed with the NIR spectra obtained on the slave instrument, and the predictive ability of the resulting model was equal to the one of the model developed with the data collected with the internal measurement cell. For longer fibre optic devices, much more noisy NIR spectra may be obtained (particularly in the 2200–2500 nm region) and different conclusions might be obtained. In such a case, the standardisation of both measurement modules might also improve the predictive ability of the model developed with data collected with the fibre optic probe, as in the case where two instruments of different qualities must be standardised [33].

Table 1
Standard errors of predictions for the pirisudanol concentrations (in mg g^{-1}) determined with the NIR spectra collected with the fibre optic module, without standardisation and with standardisation using either the slope/bias correction method or the Shenk–Westerhaus algorithm

Standardisation method used	No Standardisation	3	4	5	6
Slope/bias correction	53.2	8.8	8.3	8.1	8.3
Shenk–Westerhaus	53.2	10.3	7.4	7.7	7.9

Standardisation was performed using three to six standardisation samples.

5. Conclusion

Standardisation methods have been applied to a pharmaceutical problem, in which a quantitative analysis of tablets must be performed by a NIR spectrometer equipped with a fibre optic probe. The use of standardisation techniques enabled to correct spectral differences between the internal measurement cell and the fibre optic module, and therefore allowed to scan routine samples with the fibre optic module, and still obtain satisfactory predictions with the calibration model developed with spectra scanned with the internal measurement cell. The ability of standardisation methods to correct for differences between measurement modules can be very useful for some recent applications of NIR spectroscopy in the pharmaceutical industry, particularly for on-line applications using fibre optics devices.

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