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Short communication

Augmentation of near infrared diffuse reflectance and transmittance spectral data for the development of robust PLSBC models for classifying double blind clinical trial tablets

T. Van den Kerkhof*, R. De Maesschalck, K. Vanhoutte, M.C. Coene

Janssen Pharmaceutica NV, Pharmaceutical Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium

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Abstract

The water content of clinical trial tablets can be different between and within different tablet batches, depending on the relative humidity conditions during their production, packaging, storage and analysis. These water variations lead to important spectral variations in the near infrared spectral region which can lead to a wrong identification if the classification model was based on unrepresentative data towards the water content. As model development for clinical trial studies needs to be extremely fast – within one working day – with generally only one batch available, the principle of data augmentation has to be applied to render more robust classification models. Therefore, tablets available for constructing the model are being processed in order to increase or decrease their water content and to make them more representative for tablets to be tested in the future. The inclusion of a deliberate water variation is the most efficient way to develop a model, for which no additional model redevelopment will be required to pass the system suitability tests and to obtain a correct identification.

Keywords: Clinical trial studies; NIR; PLSBC; Data augmentation

1. Introduction

Near infrared spectroscopy (NIR) in transmission or reflectance mode has been successfully applied in the pharmaceutical industry for several applications such as e.g. the end-point determination of a hydrogenation reaction [2] or a drying process [3,4], blend homogeneity [5–7], content uniformity [8,9] and polymorph determination [10,11]. In a previous article [1] we described the PLSBC approach for using NIR spectroscopy as identification test of blister packed double blind clinical trial tablets. The largest benefit of NIR spectroscopy for this application is its speed. The classical way for identifying the tablets is to apply UV spectroscopy or liquid chromatography, which usually requires dissolving and filtering the sample. This procedure is rather time-consuming since, including sample preparation, it can take up to 30 minutes cycle time. NIR measurements need no sample preparation. One measurement can be performed in seconds up to minutes depending on the type of NIR instrument and its settings. To make a fair comparison, also the time invested into developing and validating the method must be taken into account. For the identification with UV spectroscopy or liquid chromatography, no special method has to be developed, since existing methods, e.g. content uniformity, can be applied. For NIR spectroscopy, a new method needs to be developed and validated. Concerning cycle time, NIR becomes more efficient as the number of samples to be measured increases. For double blind clinical trial tablets, the number of samples to be tested for the clinical trial route of one particular drug generally ranges from 800 to 12,000 tablets. This is still a relatively small amount of samples and therefore it is important to limit the time for method development and validation. In that way, the result of using NIR instead of UV spectroscopy or liquid chromatography can be a substantial gain in time or on the other hand an improvement of quality as more tablets can be tested with the same resources.

For NIR spectroscopy, identification methods can be developed by applying the PLSBC approach, which is based on partial least squares (PLS) regression combined with β-error driven class boundaries. The approach was shown to be easy to develop

* Corresponding author. Tel.: + 32 14 60 37 03; fax: +32 14 60 50 34.
E-mail address: tvdkerkh@prdbe.jnj.com (T. Van den Kerkhof).

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and validate [1]. A typical method can be developed and validated within one working day, which is very fast. Next to the speed of the development, it is even more important that the method is robust, meaning that the risk for misclassification of samples is virtually zero. The PLSBC approach was developed in order to limit the probability for misclassification as much as possible by constructing the class boundaries in function of the β-error. The probability of misclassification however does not only depend on the type of classification model and the class boundaries that are used. Another parameter which has a very large impact on the robustness of the classification, is the representativeness of the data applied for building the classification model (training set).

To obtain a robust classification model for the identification of clinical trial tablets, NIR spectra representing all tablets to be tested in the future must be available. At the time the model has to be developed, generally only one batch is available for constructing the classification model. As a result, the model may not be robust towards additional variation which may be present in newly manufactured tablet batches. In this paper, the effect of relatively small changes between batches of the same formulation towards misclassification is investigated over a large time frame and several batches. It is also shown how one can obtain robust classification models using only a limited number of tablets of one batch.

2. Experimental

2.1. Samples

The samples of two clinical studies, for investigating oral immediate release tablets of galantamine and topiramate, were studied. The galantamine tablets used in this work are coated circular tablets with a total weight of 250 mg and were manufactured to contain 4, 8 and 12 mg of galantamine, or to be a matching placebo. These tablets were measured in transmission mode. In order to introduce additional variation between tablets of one batch, several tablets are being processed by storing them in a different environment before measuring. To increase the water content of the tablets, they were stored at 75% relative humidity for 3 h. Storage at 50 °C for 30 min was used to decrease their water content. These storage conditions were chosen in such a way that the total weight difference of the tablets between the conditions was approximately 5 mg or 2% (w/w).

The topiramate tablets are white layered coated oblong tablets with a total weight of 319 mg and were manufactured to contain 25 mg of topiramate, or to be a matching placebo. These tablets were measured in the diffuse reflection mode. Different water contents were achieved in the same way as for the galantamine tablets. The weight difference between the two conditions was 5 mg or 1.6% (w/w).

2.2. Instrumentation and software

The FT-NIR spectra were recorded on a Bruker Vector 22/N-T spectrometer, using a Tungsten Halogen source in combination with a Quartz beamsplitter and an InGaAs detector. The interferograms were recorded with a resolution of 8 cm^{-1}, averaged over 16 (reflection) or 64 (transmittance) scans, Blackman-Harris 3-Term apodized and Fourier transformed with a zero filling factor of 2.

Calibration models were built using the Quant 2 software package version 4.2, an add-on to the general OPUS spectroscopy software version 4.2. Quant 2 contains the PLS regression technique needed for the PLSBC approach. Setting the class boundaries was performed by using a validated Excel sheet (Microsoft).

3. Discussion and results

The sources of variation within NIR spectra of tablets of the same theoretical composition are physical parameters such as the particle size, particle morphology and density (tablet compression force). These parameters generally can be eliminated from the spectra by applying a suitable spectral pre-treatment method such as e.g. standard normal variate (SNV) [12] or first and second derivative [13]. The excipients in the tablet or the coating can vary slightly between different batches or samples (excipient homogeneity). Although mostly not relevant in vivo, these factors can have an influence on the NIR spectra. In practice, the use of a minimal amount of tablets of one batch is enough to include this variation into the model since the manufacturing of batches is very consistent. To assure that no significant additional variation is included in the spectra of newly produced batches, a system suitability test (SST) is performed. The SST includes the prediction of a few tablets of each new batch with a known identity by using the model. If misclassification should occur, the model must be redeveloped by including a suitable number of NIR spectra of the new batches and be revalidated. As will be shown in this paper, this situation almost never occurs in practice.

A much more important parameter which is often adding a lot of additional variation and which has not been fully covered by the calibration tablets, is the water content of the tablets. For most tablets, water can be taken up very fast depending on the relative humidity of the environment where the NIR measurements are performed. Fig. 1 shows the increase and decrease of water in a topiramate and galantamine tablet as a function of the relative humidity. By storing these tablets in a high relative humidity environment, a substantial increase of the water content can be achieved in a very short period. Even in blisters, the tablets can contain a different amount of water due to the fact that mostly in the packaging facilities often no or only limited humidity control is used. Small amounts of water present in the tablets lead to very strong and broad absorbance bands at 7300–7000 (first overtone of O–H stretch) and 5300–5000 cm^{-1} (combination band of O–H stretch and O–H deformation) in their NIR spectra, which is demonstrated by the topiramate tablets in Fig. 2. First derivative spectra are calculated in order to remove the baseline offsets and to make the spectral regions, which undergo a change, more visible. The spectral variations due to the drug and water content of the topiramate tablets are shown in Fig. 3. Next to the water variations, spectral changes due to the pres-

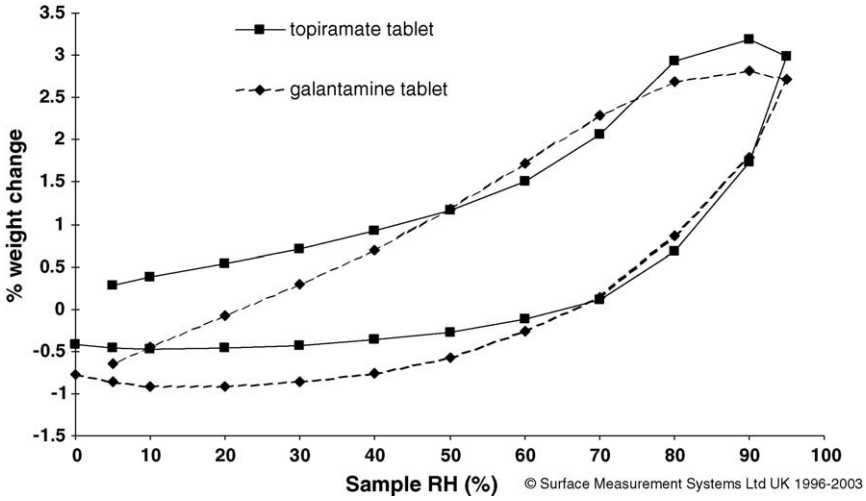

Fig. 1. Adsorption–desorption curve of a topiramate tablet and a galantamine tablet.

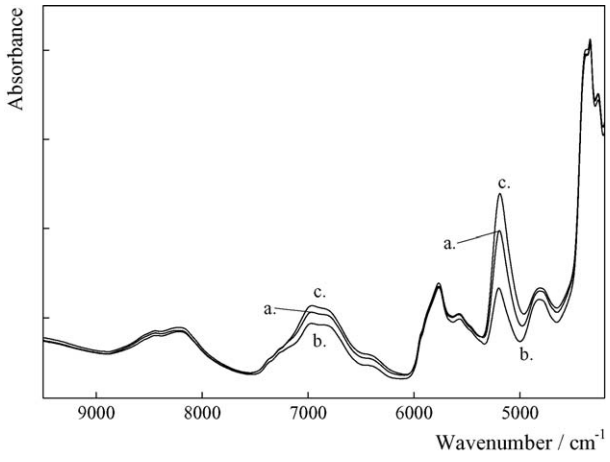

Fig. 2. Near infrared reflectance spectra of a 25 mg topiramate tablet. (a) Recorded under normal circumstances; (b) after storage at 50 °C for 30 min; (c) after storage at 75% relative humidity for 3 h.

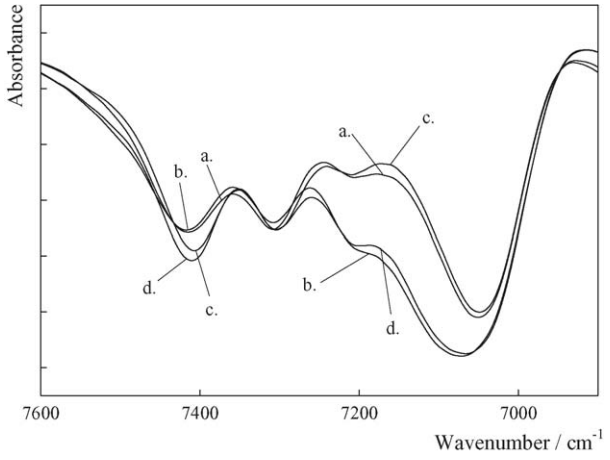

Fig. 3. First derivative of the near infrared reflectance spectra of topiramate tablets. (a) Placebo, recorded after storage at 50 °C for 30 min; (b) placebo, recorded after storage at 75% relative humidity for 3 h; (c) 25 mg, recorded after storage at 50 °C for 30 min; (d) 25 mg, recorded after storage at 75% relative humidity for 3 h.

ence or absence of topiramate can be observed in the regions at 7500–7300 and 6100–5800 cm^{-1}. Traditionally, a classification model is built, using the PLSBC approach, on one batch of tablets without processing them to different water contents. Therefore, a good way to test the robustness of the achieved model is to use it to predict the identity of the "processed" tablets. Using the full spectral range (10,000–4500 cm^{-1}) of 20 topiramate tablets per class, the best calibration model was obtained after calculating the first derivative of the spectra and using six PLS components (Table 1). The average prediction values, by the model, for the processed tablets are shown in Table 2. Although no misclassifications occur, the obtained model is certainly not always able to confirm the identity of the processed tablets. Some tablets are not inside any of the class boundaries and are therefore left unassigned. The concentration of topiramate in the tablets with a higher water content is being overestimated by the model. At the same time, the predictive ability of the model appears to be sufficient for tablets with a lower water content. As the drug dependent spectral variations do not coincide with the variations due to the water content, a classification model can be built by leaving out the spectral regions of water. The best model, for the spectral region 10,000–7300 and 6440–5600 cm^{-1}, is obtained after calculating the first derivative of each spectra and using five PLS components (Table 1). Now, the obtained PLSBC classification model is able to confirm the identity of every processed tablet. Despite their positive identification, the predictive ability of the model is rather low, especially for spectra of tablets which contain a lower amount of water. This can be expected because the absorbance of water is not limited to the spectral regions 7300–7000 and 5300–5000 cm^{-1}, but that it influences the entire wavelength range of the diffuse reflectance spectra. Applying only a system suitability test as described, is not recommended since the model would then be the subject of constant remodelling.

To avoid the need to update the models each time a new batch is produced, the principle of data augmentation [14] can be applied. Data augmentation can be defined as the action of intentionally introducing additional information into the data by simulation or adding synthetic samples. This should make

Table 1
PLSBC classification models for the identification of clinical trial topiramate tablets

	Model 1	Model 2	Model 3
Spectral region (cm^{-1})	10000–4500	10000–7300 and 6440–5600	10000–4500
Data pre-treatment	1st derivative	1st derivative	1st derivative
# PLS components	6	5	8
Training set	20 spectra/class no processed tablets	20 spectra/class no processed tablets	40 spectra/class + processed tablets
Classification	−5.94 < 0 mg < 6.09 18.85 < 25 mg < 30.96	−6.61 < 0 mg < 6.71 17.60 < 25 mg < 32.14	−7.63 < 0 mg < 7.76 17.01 < 25 mg < 32.78

the data more representative for future batches still to be produced. Therefore, randomly half of the spectra of the processed tablets are added to the training set, while the remaining spectra are used for validation. Again, using the full spectral region (10,000–4500 cm^{-1}) of 40 spectra per class, the best classification model is obtained after calculating the first derivative of the spectra and using eight PLS components (Table 1 — model 3). Averaged prediction results of the spectra used for validation are shown in Table 2. Next to the positive identification, the results also show the accurate predictive ability of the calibration model. When including the variation of water in the different classes, the PLS algorithm will find no correlation between the concentration of active in the tablets and the variation in the spectra due to the presence of different amounts of water. By excluding this correlation, the model becomes insensitive towards the water content and therefore the risk of misclassification is decreased and the model is rendered more robust.

Contrary to reflectance spectra, the two water bands cannot be observed in transmittance spectra, due to the smaller applicable spectral range. As the absorbance of water influences the entire spectral region, the principle of data augmentation may also be considered for classification models based on transmittance spectra. The effect of water on the transmittance spectra of galantamine tablets is shown in Fig. 4. To make the effect more visible, second derivative spectra are calculated. Fig. 4 shows that the effect of water is the most obvious in the spectral region 8800–8600 cm^{-1}, which partly coincides with the spectral variations due to the experimental drug galantamine (Fig. 5). The best classification model without data augmentation, is obtained after taken the standard normal variate (SNV) of each spectrum in the region 10000–8700 cm^{-1} for 20 spectra per class and using seven PLS components (Table 3). Although no misclassifications are obtained, the model misses the ability to identify all processed tablets. In general, tablets processed with a lower water content are predicted to contain a slightly higher galantamine concentration than their real concentration, but this prediction is still good enough to obtain a positive identification. The prediction of the tablets with a high water content is not always sufficient for a positive identification, as the model tends to underestimate their galantamine concentration. The principal of data augmentation is again applied by randomly adding half of the spectra of the processed tablets to the training set and using the remaining spectra for validation. By using the same spectral region, data pre-treatment and number of PLS components a PLSBC classification model with data augmentation

Table 2
Averaged prediction values and their standard deviation of the processed topiramate tablets by the different classification models

Condition	Model 1	Model 2	Model 3
0 mg–30′ 50 °C	1.56 (2.26)	−2.19 (1.42)	0.70 (1.02)
0 mg–3 h 75% rh	6.95 (1.05)	1.90 (0.92)	0.32 (0.99)
25 mg–30′ 50 °C	24.64 (1.75)	20.65 (1.27)	24.85 (1.03)
25 mg–3 h 75% rh	29.85 (0.99)	24.69 (1.12)	25.02 (0.72)

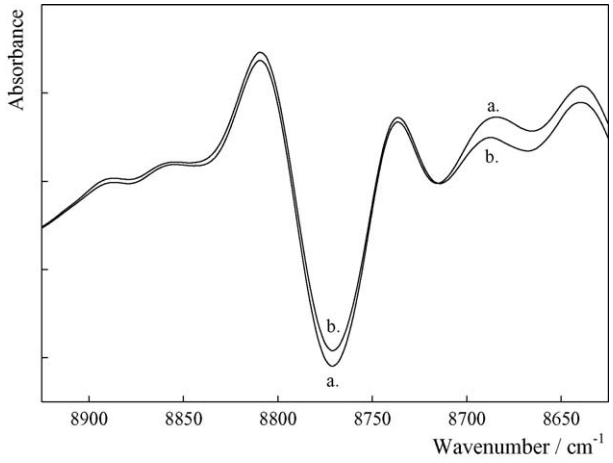

Fig. 4. Second derivative of the near infrared transmittance spectra of a 4 mg galantamine tablet. (a) after storage at 50 °C for 30 min; (b) after storage at 75% relative humidity for 3 h.

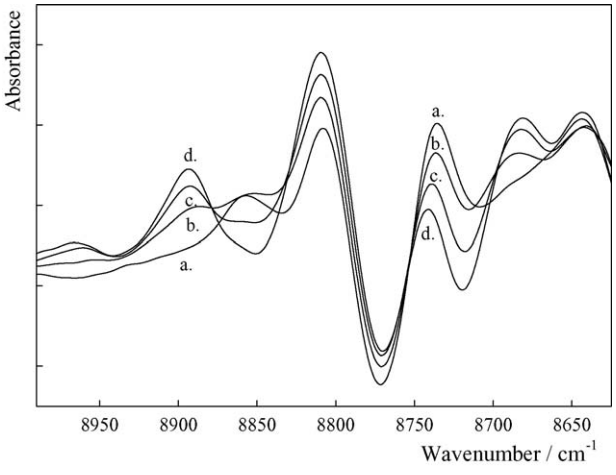

Fig. 5. Second derivative of the near infrared transmittance spectra of galantamine tablets. (a) placebo; (b) 4 mg; (c) 8 mg; (d) 12 mg.

Table 3
PLSBC classification models for the identification of clinical trial galantamine tablets

	Model 1	Model 2
Spectral region (cm^{-1})	10000–8700	10000–8700
Data pre-treatment	SNV	SNV
# PLS components	7	7
Training set	20 spectra/class no processed tablets	40 spectra/class + processed tablets
Classification	−0.185 < 0 mg < 0.187 3.610 < 4 mg < 4.414 7.472 < 8 mg < 8.480 11.342 < 12 mg < 12.674	−0.374 < 0 mg < 0.370 3.528 < 4 mg < 4.500 7.451 < 8 mg < 8.519 11.163 < 12 mg < 12.843

Table 4
Averaged prediction values of the processed galantamine tablets by the different classification models

Condition	Concentration (mg)	Model 1	Model 2
30′ 50 °C	0	−0.021 (0.078)	−0.084 (0.029)
	4	4.058 (0.054)	3.948 (0.054)
	8	8.119 (0.070)	8.000 (0.054)
	12	12.420 (0.135)	12.003 (0.029)
3 h 75% rh	0	−0.215 (0.057)	−0.120 (0.039)
	4	3.501 (0.094)	4.023 (0.046)
	8	7.407 (0.090)	8.032 (0.075)
	12	11.468 (0.099)	11.945 (0.099)

was built (Table 3 — model 2). Table 4 shows the very accurate predictive ability of the data augmented model. Now, all tablets are being correctly identified with a very accurate prediction. Although spectral variations due to variations in the water content are generally not visible in transmittance spectra, they still have a big influence and therefore it's of the greatest importance to take these variations into account during method development, so that a robust classification model is achieved.

In practice 12 PLSBC models, developed with the data augmentation principal, are up and running for an average time of 15 months. Although, for most products only one batch for each class was available for method development, for some products two batches could be used for method development. Each time a newly produced batch is used in the clinical trial study, a system suitability test is performed by confirming the identity of minimum six release samples of the newly produced batch. Table 5 shows for each PLSBC model, the number of analysed samples and the number of performed system suitability tests. During the 15 months that the methods are running, 83 system suitability tests were performed. The system suitability test failed for two batches. Although no misclassification occurred for these batches, the corresponding PLSBC model was not able to confirm the identity of these samples. Their predicted values were outside the class boundaries and the samples were therefore left unassigned. The corresponding PLSBC models were updated by merely adding the six new spectra to the calibration model (training set). After this update, the method needs to be revalidated. This revalidation is performed by reanalysing the spectra of the first validation and analysing the spectra of six additional measured samples of the newly produced batch.

Table 5
The performance and system suitability tests for the PLSBC models used over a 15-month period

Product	# Classes	# Samples	# SST (new batches)	# SST fails	# Method updates
Risperidone	6	1592	19	0	0
Galantamine	4	394	9	1	1
tmc125	2	1453	16	0	0
tmc114	4	1548	8	0	0
Dapoxetine	4	410	6	0	0
Topiramate	2	404	3	1	1
Other methods	–	1826	22	0	0

4. Conclusions

Many sources of spectral variations within NIR spectra of tablets of the same theoretical composition can be accounted for by using a minimal amount of spectra of one batch and by applying the adequate data pre-treatment. As generally only one tablet batch is available for model development, a system suitability test is performed to assure there's no significant additional spectral variation present in the spectra of the newly produced batches.

A source of spectral variation that has a major impact on the NIR reflectance and transmittance spectra and on the classification model is the water content of the tablets. As the time and samples for method development are limited, it's necessary to apply the principle of data augmentation in order to make the classification model more robust for these water variations. Therefore, during method development, several tablets need to be processed to contain a different water content and to make them more representative for future tablets. Inclusion of the water variations in the model will exclude a possible correlation between the water spectral variations and the concentration of active and renders a more robust model with a more accurate predictive ability.

The principle of data augmentation, for the deliberately introduced water variations in the tablets, and the system suitability tests, performed for each newly produced batch, ensure an efficient and robust process for the method development and routine analyses for the classification of double blind clinical trial samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2006.05.007.

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