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PAPER

A new and simple PLS calibration method for NIR spectroscopy. API determination in intact solid formulations†

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The NIR spectra of pharmaceuticals are the result of component contribution and the effects of the different steps of the production process (granulation, compaction, and coating). These effects, even though they are of low magnitude, affect significantly the results of calibration models. Incorporating such effects into the calibration set is essential with a view to constructing a model capable of accurately predicting the contents of production tablets. We developed a new method for incorporating the variability introduced by the production process in the spectra for the calibration set. The method calculates the difference between the spectrum of a tablet and a powder mixture of identical composition prepared in the laboratory; the differences thus calculated for several tablets constitute a set of mathematical vectors that define the overall variability matrix of the process: the *process variability matrix*. This matrix is added to a set of NIR spectra for several powder mixtures (prepared in laboratory) spanning the desired content range for the active pharmaceutical ingredient (API) in order to obtain the spectral matrix for the calibration set. The API content (in %) of calibration samples is established from weights of their components in the laboratory powder mixtures. The calibration model is constructed by applying the partial least-squares (PLS) algorithm to the spectral matrix of the calibration set. This methodology has been applied successfully on API determination in commercial pharmaceutical tablets.

Introduction

By virtue of its simplicity and expeditiousness, and the quality of its results, near infrared (NIR) spectroscopy has over the last few years become the spectroscopic technique of choice for the pharmaceutical industry. However, obtaining accurate results with this technique requires use of an appropriate calibration set containing all possible sources of variability in the production samples for each formulation. Also, constructing the calibration model entails using an appropriate reference method.

The production process of pharmaceuticals involves a series of unit steps by which the starting raw materials are subjected to various treatments of primarily physical nature that alter the behaviour of the formulation components and also their NIR spectra.

The production of solid formulations, which are the most frequent, involves blending their components, usually in powder form, to obtain a homogeneous mixture. The powder mixture is then granulated to increase the particle size in order to avoid disaggregation of its components during transport, and also to improve compaction and sliding properties in the mixture. Therefore, the median particle size and measurement of the cumulative particle size distribution are critical parameters that have been tackled by NIR spectroscopy.[1,2]

The granulated, homogeneous mixture is then pressed at an appropriate pressure to obtain hard enough tablets to resist crumbling and fracture, but also soft enough to facilitate disaggregation in the body once swallowed. The NIR spectrum is influenced by the compaction pressure; this has led to several studies to determine it, and even the dissolution test measurements by NIR.[3] In other studies, the applied pressure has been taken into account to ensure the success of API determination by NIR.[4]

The final cores thus obtained are often coated with a thin film of polymers in order to protect them from the environment, conceal unpleasant flavours, or facilitate controlled release of the API.[5] The reduced thickness of the coating film does obtain the spectrum of the compounds of the core. The coating thickness measurements have also been investigated by NIR spectroscopy,[6,7] in these cases NIR analysis is alternatively much faster and cheaper than scanning electron microscopy (SEM).

The starting powder mixture undergoes various physical changes such as an increase in the particle size, pressing into a preset shape and potential polymorphic transformations, during the production process; in addition, it is coated with a film that may affect release of the API in the body. These changes

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introduce alterations that are somehow reflected in spectra obtained after each step of the process, and hinder the construction of an appropriate calibration set and accurate prediction of their effects.

A number of studies have been devoted to find ways of incorporating the spectral changes caused by the different steps of the production process into calibration sets. This can be accomplished mainly in two ways, namely: (a) by mimicking the production process in the laboratory[8] or (b) by including production samples in the calibration set.[9] The former approach is confronted with some difficulties arising from the difficulty of incorporating all possible sources of variability in a pharmaceutical process into a laboratory experiment. In contrast, the latter is easy to implement since it only requires using some production samples with a known value for the target property, so it is the most widely used.

In this work, we developed a new method for incorporating the variability introduced by the production process in order to obtain NIR spectral sets containing pertinent information about both the different steps of the process (granulation, compaction and coating), which introduce variability in the pharmaceutical process, and the chemical composition of the formulation concerned (pharmaceutical chemical information). The variability of the production process is collected in a process variability matrix that is used to incorporate the variability generated in each process step into a set of powder samples containing the chemical variability of the formulation in order to obtain an appropriate sample set (total variability matrix) for constructing the PLS calibration model required to determine the API in production tablets. This total variability matrix is characteristic for each pharmaceutical formulation and for the particular production process used, because it embodies the possible interactions between the different components (API and excipients) as well those caused by the different steps of the production process (granulation, compaction, coating, *etc.*)

Experimental

Production samples

The pharmaceutical formulation studied contains a high proportion (89.29 wt%) of API (metformin hydrochloride) and relatively low proportions (8.41 wt%) of three excipients (hydroxypropylmethylcellulose, polyvinylpyrrolidone and magnesium stearate). The coated film, which consists of hydroxypropylmethylcellulose, macrogol 6000 and titanium dioxide, accounts for 2.31 wt% of each tablet.

The tablets have an oblong shape and have a score on each side, and are biconvex and coated with a white lacquer on both sides. The lower side has a wide score (WS) and the upper side a narrow score (NS) (see Fig. 1).

The tablets were collected from a total of 13 production batches. All were kindly supplied by KERN PHARMA, S.L. (Terrassa, Spain).

Laboratory samples

A total of 30 laboratory samples were prepared by mixing accurately weighed amounts of their components, in powder form, spanning a content range ±20% around the nominal API value (ICH guideline);[10] however, the content range spanned was in fact 80–112% API, the upper limit corresponding to a sample containing pure API. A mixture containing the nominal concentrations of excipients was also prepared by weighing and added to different amounts of API in order to obtain new mixtures with the API spanning the previously defined content range.

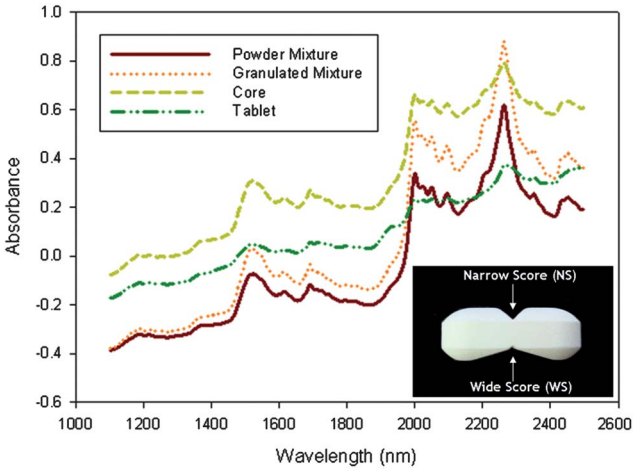

Fig. 1 Spectra of laboratory powder mixture, granulate mixture, core and production tablet. All sample components were at their nominal concentrations. Inset: an image of a commercial tablet.

All samples were prepared by weighing the mixture components on an analytical balance and homogenization in a solid blender. The mixtures were assumed to be homogeneous when they gave two identical consecutive NIR spectra. All concentration values are given as percentages of the API nominal content.

Instrumentation and software

Laboratory samples were homogenized in a Turbula T2C WAB shaker mixer and their spectra recorded on a Foss NIRSystems 6500 spectrophotometer equipped with a Rapid Content Analyser (RCA) module governed with the Vision v. 2.5.1 software package, also from Foss NIRSystems.

Spectra were processed and multivariate models, Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression, were constructed by using the software The Unscrambler v. 9.8 (Camo Process, Oslo, Norway).

Recording of NIR spectra

Near infrared reflectance spectra for the powder samples were recorded in glass cells about 3 cm in diameter that were placed on the window of the RCA module. Before each measurement, a reference spectrum of the glass cell was taken. A total of 3 spectra per sample were obtained with turnover between recordings in order to calculate an average spectrum for each sample.

The spectra for the tablets were obtained by placing them on the instrument window and adjusting the shutter aperture. A duplicate spectrum for each tablet side was recorded to obtain an average value.

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Construction of the process variability matrix and calibration/validation set

The spectral variability S_P for each tablet and tablet side was calculated from eqn (1),

$$S_P = S_T - S_{100\%\ lab} \quad (1)$$

where S_P is the process spectrum, S_T that for a production tablet and $S_{100\%\ lab}$ that for a laboratory powder sample containing the nominal concentrations of API and excipient (the reference spectrum).

Two different S_T spectra per tablet were recorded: one for the WS (wide score) side, S_{PWS} and the other for the NS (narrow score) side, S_{PNS} (see Fig. 1). Three different tablets from each production batch were used to obtain as many process spectra, S_P, those were averaged into a single variability spectrum for each side. The procedure was applied to three different batches, so a total of three spectra for each side (S_{PWS} and S_{PNS}) were obtained. This body of spectra constituted the *reduced process variability matrix* (S_P). The spectra in this matrix were randomly added to those for the 30 powder samples in order to construct the *total variability matrix* (S_{var}), which encompassed 30 WS samples and 30 NS samples. Such a matrix was split into a calibration set containing 15 WS samples and 15 NS samples, and a validation set containing all others.

$$\boldsymbol{S}_{\mathbf{var}} = \boldsymbol{S}_{\mathbf{lab}} + \boldsymbol{S}_{\mathbf{P}} \quad (2)$$

In order to increase the variability of the production process, S_P was multiplied by either 0.5 or 1.5. This provided six spectra with factor 0.5 (three $S_{PWS\ 0.5}$ and three $S_{PNS\ 0.5}$) and another 6 with factor 1.5 (three $S_{PWS\ 1.5}$ and three $S_{PNS\ 1.5}$). The 18 process spectra thus obtained (three $S_{PWS\ 0.5}$, S_{PWS}, $S_{PWS\ 1.5}$, $S_{PNS\ 0.5}$, S_{PNS} and $S_{PNS\ 1.5}$ each) constituted the *extended process variability matrix* and were randomly added to those for the laboratory powder samples (eqn (3)):

$$\boldsymbol{S}_{\mathbf{var.ext}} = \boldsymbol{S}_{\mathbf{lab}} + \boldsymbol{S}_{\mathbf{P.ext}} \quad (3)$$

where $S_{var.ext}$ is the spectrum for a powder sample with the process variability, S_{lab} that for a laboratory powder sample and $S_{P.ext}$ one of the process variability spectra. The body of $S_{var.ext}$ constituted the *extended total variability matrix*.

UV reference method

The average content for a sample batch was determined by using 20 ground tablets to accurately weigh 112 mg of powder (equivalent to 100 mg of API) and transferring it quantitatively to a 100 ml calibrated flask which was then supplied with 70 ml of water, stirred mechanically for 15 min and made to volume. The solution was filtered and the first 20 ml discarded. Then, 10 ml of filtrate was diluted to 100 ml and dilution repeated (10 ml of diluted solution to a 100 ml final volume). The API concentration thus obtained should be *ca.* 0.01 mg ml^{-1}.

The standard solution was prepared by accurately weighing 40 mg of API standard in a 200 ml calibrated flask, dissolving it with water and making to volume with the same solvent. Finally, a 5 ml aliquot of the solution was diluted to 100 ml in order to obtain an API concentration of *ca.* 0.01 mg ml^{-1} in the standard.

The absorbance at 232 nm of the sample and standard was measured in a quartz cell of 1 cm path length against water as blank. The UV method has been validated following the requirements of EMA.[10]

All concentrations are given as percentages with respect to the formulation nominal value, which was taken to be 100%.

Construction of calibration models

Multivariate PLS calibration models were constructed by cross-validation, using the leave-one-out method.

The API content range spanned by the model was 80.0–112% (actual content *vs.* nominal content). Various spectral treatments and wavelength ranges were tested and the best results found to be those provided by second-derivative spectra (applying Savitzky–Golay algorithm and 11-point moving window) and the whole wavelength range (1100–2500 nm).

The optimum number of factors as regards prediction ability was taken to be that resulting in the lowest possible root mean square error (RMSE, eqn (4)):

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{m}\left(Y_i^{NIR} - Y_i^{REF}\right)^2}{m}} \quad (4)$$

where Y_i^{NIR} is the NIR-predicted API content, Y_i^{REF} that obtained by weighing and m the number of samples.

Results and discussion

The predictive ability of a PLS model improves with increasing similarity between calibration and production samples. Ideally, a calibration set should contain production samples spanning the whole desired content range. In pharmaceuticals, however, the API content rarely differs by more than ±5% from the nominal value; such a narrow range precludes the construction of robust, accurate prediction models and must be expanded in some way. Usually expands the range of concentrations in ±20% from the nominal value to ensure the implementation of the model in uniformity of content studies. In this work, we used a novel, very simple approach to constructing the calibration set for the NIR analysis of pharmaceuticals.

Because each step of the production process increases the variability of the spectrum for the end-product, the total variability of the process can be assumed to be the addition of those for its individual steps. This variability is often quite small, but they can have a strong effect on calibration models and their ability to accurately predict the API contents of production samples. Fig. 1 shows the NIR spectra obtained in each step of the production sample, from a laboratory powder sample to the coated tablet; both the API and the excipients were at their nominal concentrations in all samples. A comparison of the results for the powder sample and granulate mixture reveals a change in the baseline slope which can be ascribed to the difference in the particle size between the two types of sample. Also, compaction of the powder reduces the baseline slope and causes a slight flattening of the bands in the spectrum for the core (see, for example, the band around 2300 nm). The baseline of the spectrum for the coated

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tablet also exhibits a lower slope than that for the core, in addition to weaker bands by effect of its coating. The contribution of each step of the process to the NIR spectrum can be determined by calculating the differences between the spectra for consecutive steps (Fig. 2A). Thus, the impact of granulation can be assessed from the difference between the spectra of the granulate and a powder sample with identical concentrations of API and excipients ($S_{granulation} = S_{granulate} - S_{100\%}$). Similarly, the effect of compaction can be calculated as $S_{compaction} = S_{core} - S_{granulate}$ and that of coating as $S_{coating} = S_{tablet} - S_{core}$. The overall variability of the process will be the combination of $S_{granulation}$, $S_{compaction}$ and $S_{coating}$. Fig. 2B compares two process spectra obtained in two different ways: $S_{process1} = S_{granulation} + S_{compaction} + S_{coating}$ and $S_{process2} = S_{tablet} - S_{100\%\ lab}$. Both are essentially identical; therefore, process spectra can be obtained from the second equation, which is much easier to solve (see Section "Construction of the process variability matrix"). As can also be seen from Fig. 2B, the region from 1100–1900 nm contains a virtually constant variability of *ca.* +0.2 a.u.; on the other hand, the region from 1900 to 2500 nm includes variable, more marked differences (*ca.* −0.2 a.u.), especially in the zones exhibiting the strongest bands (2300 nm), which are the result of the multiplicative effect of scattering. In summary, the effect of variability in the production process is greatest in those spectral regions exhibiting the highest absorbance.

All process spectra show the same shape but there is an intensity change between tablets due to variations in granulometry and distribution of components, in compaction pressure, in film thickness of coating, *etc.*; these facts explain the observed differences between tablets. The different intensities of these contributions explain the different values of the process spectra; for these reasons we expand the variability process spectra applying factors near to one to initial process spectra. These total variability process spectra are also added to spectra of laboratory samples in order to span the whole variability matrix. In this particular case, in order to incorporate the potential effects of differences in geometry between the two sides of tablets (see Fig. 1) we recorded NIR spectra for each tablet side and subsequently, the process spectra were calculated for each side.

The calculations described in Section "Construction of the process variability matrix" provided various process spectra that were randomly added to those for laboratory powder samples spanning the content range stated under the Experimental section in order to obtain a new set of spectra.

Fig. 3 is a scatter plot of the PCA scores for laboratory samples added with process spectra and also for production samples. The spectra were SNV-treated and spanned the whole wavelength range (1100–2500 nm). As can be seen, the cluster of laboratory samples included virtually all production samples; therefore, modified laboratory samples incorporate most of the variability introduced by the production process.

The modified laboratory samples were split into two sets; one was used to construct the PLS calibration model and the other to assess its predictive ability. Table 1 shows the figures of merit for model NIR100. This model is extremely simple; in fact, 2 PLS factors sufficed to account for 98.1% of the samples variance, and the calibration and prediction RMSE values were not

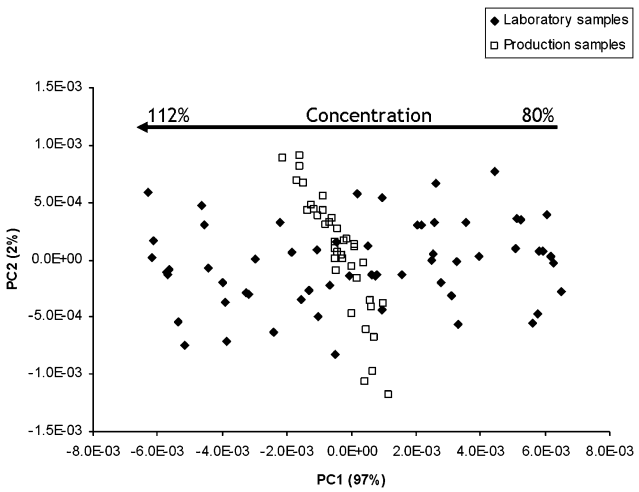

Fig. 3 Scatter plot of PCA scores for laboratory samples modified with spectra for the process and production samples. Spectral mode: second-derivative. Wavelength range: 1100–2500 nm.

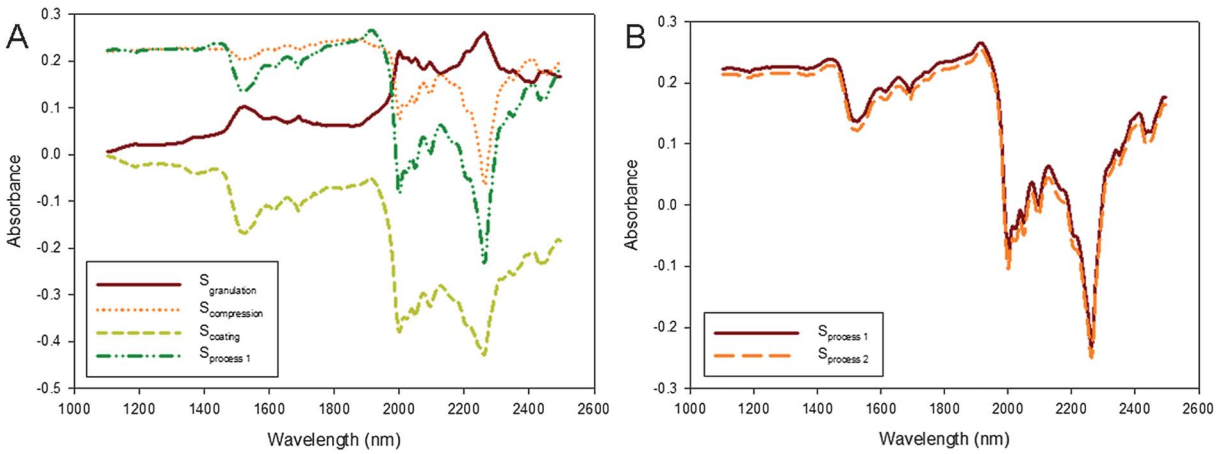

Fig. 2 Process variability spectra. (A) Effects of granulation ($S_{granulation} = S_{granulated} - S_{100\%\ lab}$) and compaction ($S_{granulation} + S_{compaction} + S_{coating}$). (B) Comparison of process spectra obtained by adding up the effects of each step ($S_{process1}$) with the difference between those for a tablet and a powder mixture ($S_{process2} = S_{tablet} - S_{100\%\ lab}$).

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Table 1 Figures of merit of the different models constructed[a]

	NIR100		NIR95		NIR105		NIR100 extended	
	Cal.	Pred.	Cal.	Pred.	Cal.	Pred.	Cal.	Pred.
Treatment	2nd derivative		2nd derivative		2nd derivative		2nd derivative	
Range/nm	1100–2500		1100–2500		1100–2500		1100–2500	
API content (%)	80–112		80–112		80–112		80–112	
No. of PC	2		2		2		4	
Variance Y (%)	98.1		97.6		97.8		99.2	
Offset	2 ± 5	0 ± 5	2 ± 6	0 ± 4	2 ± 6	1 ± 5	1 ± 2	0 ± 1
Slope	0.98 ± 0.05	1.00 ± 0.05	0.98 ± 0.06	1.00 ± 0.04	0.98 ± 0.06	0.99 ± 0.05	0.99 ± 0.02	1.00 ± 0.01
Number of samples	30	30	30	30	30	30	90	90
RMSEC/P	1.30	1.24	1.43	1.09	1.36	1.54	0.83	0.50

[a] NIR100, NIR95 and NIR105 are models constructed with reference to nominal, 95% and 105% levels, respectively. NIR100 extended is a model constructed using the extended variability matrix.

significantly different. The proposed PLS model (NIR100) was used to predict the API contents of 13 production batches. The value for each batch was taken to be the average of the predicted values for 10 tablets. The predictions obtained are shown in Table 2 on the NIR100 column. The reference column lists the API content in the batch as determined with the reference method. A paired *t*-test between the reference (UV) values and those provided by the NIR100 model gave $t_{calc} = 2.49$ and $t_{crit\ 0.05/2,\ n\ =\ 13} = 2.179$; therefore the two methods provided statistically different results.

Process spectra may contain a systematic error—one which will be transferred to calibration spectra—if the reference powder mixture ($S_{100\%\ lab}$) does not contain exactly the same amounts of API and excipients as the production tablets used to record the process spectrum. In order to confirm this hypothesis, the process spectrum was obtained by using a powder mixture containing an API concentration of 95.7% the nominal value and excipient concentrations identical with those in the mixture containing 100% API. The calibration and validation sets were obtained as described in Section "Construction of the process variability matrix", but using the new process spectra. Table 1 shows the figures of merit of the new PLS model (NIR95). Process spectra were obtained identically as before and PLS models constructed by using a reference sample containing 104.3% API (model NIR105). The results thus obtained are also shown in Table 1.

Table 2 shows the concentration values and the residuals obtained by subtracting the predictions of NIR95 and NIR105 from the reference values. Based on the mean residuals, Ref − NIR95 and Ref − NIR105, using the spectra for the samples containing 95.7% and 104.3% of the API nominal content as reference introduced a systematic error of −3% and +4%, respectively. Therefore, the calibration model can be expected to give a systematic error if the reference spectrum, recorded from a laboratory powder sample, does not contain exactly the same amounts of API and excipients as the end-product. Based on this conclusion, we used batches 1–3 to record the process spectra since their average API contents (reference column in Table 2) coincided with the API nominal content in the reference mixture (100.1%).

Table 2 Predictions for different production batches and residuals between models

						Residual			
	Reference	NIR95[a]	NIR100[a]	NIR105[a]	NIR100 extended[a]	Ref − NIR95	Ref − NIR100	Ref − NIR105	Ref − NIR100 extended
Batch 1	100.12	96.87	100.86	104.00	99.77	3.25	−0.74	−3.88	0.35
Batch 2	100.05	95.65	99.64	102.81	99.28	4.39	0.41	−2.76	0.77
Batch 3	100.31	95.44	99.32	102.53	99.59	4.87	0.99	−2.23	0.72
Batch 4	98.07	95.40	99.49	102.47	99.84	2.68	−1.42	−4.40	−1.77
Batch 5	99.49	95.60	99.38	102.59	100.29	3.89	0.11	−3.10	−0.80
Batch 6	99.19	98.77	102.93	105.96	99.60	0.41	−3.74	−6.78	−0.41
Batch 7	98.74	98.16	102.28	105.35	99.76	0.58	−3.54	−6.61	−1.02
Batch 8	98.07	97.16	101.14	104.21	99.31	0.91	−3.07	−6.14	−1.24
Batch 9	98.25	95.97	99.94	103.11	99.54	2.27	−1.70	−4.86	−1.29
Batch 10	100.84	96.89	100.86	104.00	99.64	3.95	−0.02	−3.16	1.20
Batch 11	101.95	98.30	102.29	105.36	99.29	3.66	−0.34	−3.41	2.66
Batch 12	101.29	97.07	101.04	104.15	99.39	4.22	0.26	−2.86	1.90
Batch 13	98.46	95.70	99.66	102.86	99.16	2.75	−1.20	−4.40	−0.70
Mean	99.60	96.69	100.68	103.80	99.57	2.91	−1.08	−4.20	0.03
SD		1.41[b]	1.54[b]	1.47[b]	0.43[b]	1.49	1.56	1.51	1.35

[a] The NIR value was the mean of the predictions for 10 tablets. [b] Weighted standard deviation.

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Constructing model NIR100 from process spectra for batches 1–3 resulted in no improved predictions, however. This led us to increase the variability of the calibration set by applying a multiplying factor of 0.5 or 1.5 to the process spectra in order to mathematically expand the variability of the production process (extended process variability matrix). As with the previous models, the extended process spectra were added to those for laboratory powder samples (see Section "Construction of the process variability matrix").

The new dataset thus obtained contained samples for which the spectra were expanded with process spectra multiplied by 0.5, 1.5 or no factor ($S_{PWS\ 0.5}$, S_{PWS}, $S_{PWS\ 1.5}$, $S_{PNS\ 0.5}$, S_{PNS}, $S_{PNS\ 1.5}$). Such a set was designated "extended process variability matrix".

Fig. 4 shows a PCA scores plot for the samples in the extended total variability matrix and production samples; the spectra were second-derivative and spanned the whole wavelength range (1100–2500 nm). As can be seen, the laboratory samples modified with the process spectra S_{PWS} and S_{PNS} failed to encompass all production samples—which are also apparent from Fig. 3. However, introducing the samples modified with the process spectra $S_{PWS\cdot 0.5}$, $S_{PWS\ 1.5}$, $S_{PNS\ 0.5}$ and $S_{PNS\ 1.5}$ allowed all production samples to be included.

The modified dataset (extended total variability matrix) was split into a calibration subset and a prediction subset. The figures of merit of the PLS model thus established, NIR100 extended, are shown in Table 1. The primary difference between this model and NIR100 was the incorporation of additional factors into the former in order to consider the variability introduced by the modified process spectra ($S_{PWS\ 0.5}$, $S_{PWS\ 1.5}$, $S_{PNS\ 0.5}$ and $S_{PNS\ 1.5}$). The predictions of model NIR100 extended for the API content of the end-product are shown under the column of the same name in Table 2. The table also gives the residuals of this model with respect to the reference values. As can be seen, the mean residual was 0.03, which is much better than the value obtained with model NIR100. A paired *t*-test between the reference (UV) values and those obtained with NIR100 extended gave $t_{calc} = 0.07$ and $t_{crit\ 0.05/2,\ n\ =\ 13} = 2.179$; therefore, the results provided by the two methods were not statistically different. Also, RMSEP for the production tablets was only 1.33%.

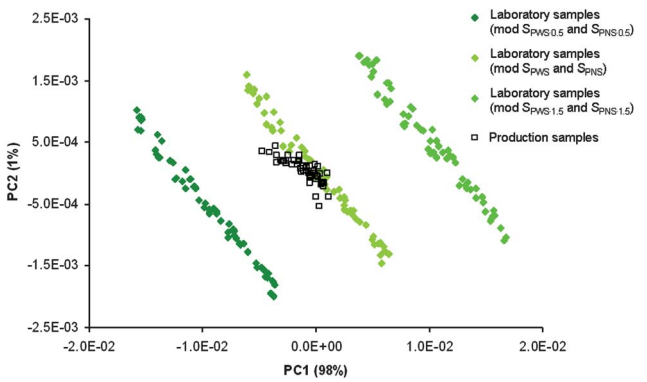

Fig. 4 Scatter plot of PCA scores for laboratory samples modified with spectra for the process ($S_{PWS\ 0.5}$, S_{PWS}, $S_{PWS\ 1.5}$, $S_{PNS\ 0.5}$, $S_{PNS\ 1.5}$) (extended process variability matrix) and production samples. Spectral mode: second-derivative. Wavelength range: 1100–2500 nm.

Finally, none of the calibration models used here include any production samples and yet model NIR100 extended allowed their accurate quantitation. In fact, simply adding the process spectrum to those for laboratory powder samples sufficed to incorporate the variability of the production process into the calibration set.

Conclusions

As shown here, the variability of a pharmaceutical production process (process variability matrix) can be determined from the difference spectrum between a coated tablet and a laboratory powder mixture containing identical amounts of API and excipients.

Using process spectra for laboratory samples containing the API at concentrations departing from the nominal value introduces systematic errors in the predictions of production samples. This requires exercising special care in choosing the reference spectra.

Expanding the process spectrum with multiplying factors (*e.g.* 0.5 and 1.5) extends the variability of the model and facilitates the construction of a PLS calibration model affording accurate prediction of production samples. The model containing the extended process variability matrix provides better results than the reduced model.

The models thus obtained require no values provided for the reference method to quantify the API in laboratory powder samples in as much as API concentrations are established by weighing. We used the UV reference here only to check the quality of the NIR results.

The proposed method provides a simple choice for constructing a calibration set incorporating the whole variability of the production process and facilitating the obtainment of a PLS calibration model capable of accurately predicting the API content of the end-product.

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