The process variability matrix: a novel approach to calculate a calibration model without reference method

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Introduction

Using NIR spectroscopy to determine the active pharmaceutical ingredients (APIs) and excipients in a pharmaceutical preparation requires the preparation of a set of calibration samples containing a known concentration of each component. This is the greatest difficulty the analyst is confronted with in analysing pharmaceutical formulations. The sample set should meet two essential requirements, namely: (a) it should span a concentration range that is wide enough to expose the prediction process to samples with abnormal concentrations, and (b) it should contain the whole variability, both physical and chemical, of the production process. Fulfilling the first requirement with production samples alone is virtually impossible since pharmaceutical processes are typically optimised to obtain a product meeting the specifications; as a result, the API and excipient concentrations rarely depart by more than ±5% from their respective nominal values[1], which is too narrow a range to ensure accurate quantitation of abnormal samples. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines[2] recommend using a range of ±20% around the API nominal value, but no similar criterion has been established for excipients[1-3]. The latter requirement is crucial to ensure accurate predictions. The greater the similarity of the calibration samples to those to be predicted in future (i.e. the production samples), the more accurate the predicted results will be. There are essentially three different ways of expanding the concentration range and incorporating the variability of the production process into the calibration set, namely[4]: (a) using pilot plant samples mimicking the actual production samples as regards API content and physical properties; (b) overdosing and underdosing production samples; and (c) using synthetic laboratory samples prepared by weighing known amounts of API and excipients to span the desired range of API concentrations.

The three approaches share a common problem: collinearity in the concentrations between analytes. Low correlation between concentrations is one of the requirements for NIR calibration sets since spectral correlation between the components of a pharmaceutical formulation is usually very high and impossible to correct. The scientific literature abounds with descriptions of sample composition designs allowing the construction of appropriate calibration sets. D-optimal designs are especially interesting for this purpose. Another important point to be considered in constructing an appropriate calibration set is the specific reference method to be used in order to determine the property to be predicted. The choice in each case will be dictated by the accuracy of the calibration results since an NIR determination with a chemometric method will never be more accurate than one with a reference method. However, Blanco *et al*.[5] successfully used NIR as an absolute technique requiring no reference methods to determine the API in tablets; they obtained reference values by weighing the formulation components on an analytical balance. This procedure avoids incorporating the error of the reference method into the NIR method and improves the overall quality of the determination as a result. Also, the absence of any need for a reference method leads to improved precision; to a level even surpassing that of common reference methods in some cases.[6]

Most studies in the scientific literature have been aimed mainly, or solely, at determining the API in a pharmaceutical formulation. Also, API content uniformity in a tablet batch, which is possibly the most important drug release related parameter, was the target for many. Excipients accompanying the API in a formulation are also very important in many cases, both as regards API bioavailability and preparation shelf-life. Although the European Medicines Agency (EMA) has issued no general rules on the quantitation of excipients in the final product, any which potentially affect bioavailability of the API should in fact be determined[1]. The excipients typically used as disaggregants (starch, cellulose), dyes or flavours are normally harmless; according to the EMA they need not be determined. However, an exceedingly large or small amount of excipients can lead to failure in, for example, a dissolution test; similarly, an unpleasant taste can lead to rejection of a whole batch. Although usually unimportant, determining the excipient content of a formulation can endow the product with an added value.

In this work, we report a new method for constructing NIR calibration sets from samples representative of the production process that does not require the use of a reference method to determine the target property.

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The proposed methodology produces a process variability matrix which incorporates the effects of the different production operations (granulation, compaction, coating) on the NIR spectrum. The process variability matrix is incorporated into the matrix of NIR spectra for laboratory samples prepared by weighing and spanning the desired concentration ranges for the API and excipients. The new matrix thus obtained is used to construct chemometric models which allow all components (API and excipients) in a pharmaceutical formulation to be determined. The proposed methodology provides very simple models of high predictive ability in terms of accuracy and precision; also, it eventually releases NIR spectroscopy from the need to use a reference method for quantitation. In addition, our methodology can be used for product control purposes at any stage of the production process. Finally, the fact that it enables the extremely easy construction of calibration models may have a considerable impact on FDA-approved process analytical technologies in the future.

Materials and Methods

Production samples

The pharmaceutical formulation studied contained a high proportion of API (63.5% w/w ibuprofen) and four excipients, namely: 28.5% (w/w) maize starch, 5% (w/w) Avicel pH 102 (microcrystalline cellulose), 2% (w/w) aerosil (fumed silica) and 1% (w/w) magnesium stearate. A granulated mixture of the API and excipients was used to prepare tablets containing 400 or 600 mg of API that differed in shape and weight but not in their API or excipient concentrations. The 400 mg tablets were cylindrical and the 600 mg tablets oblong; in addition, the latter were imprinted with the figure "600". Both were coated with an aqueous dispersion of sepifilm, sepisperse and polyethyleneglycol. The study was conducted on 10 batches each of the 400 and 600 mg tablets.

Laboratory samples

A total of 34 powder mixtures of the formulation ingredients was prepared in the laboratory by weighing the required amount of each component with an analytical balance and mixing them in a solids blender. A mixture was deemed homogeneous when it gave an identical NIR spectrum over three consecutive recordings.

The sample set was established using a D-optimal design in order to minimise collinearity between concentrations. Three additional powder mixtures containing the nominal concentrations of API and excipient were used as references to construct the calibration set.

Hardware and software

Laboratory samples were homogenised in a Turbula type T2C WAB shaker/mixer (Willy A. Bachofen AG Maschinenfabrik, Muttenz, Switzerland). Sample spectra were recorded on a Foss NIRSystems 6500 spectrophotometer (FOSS NIRSystems Inc., Silver Spring, Maryland, USA) equipped with a Rapid Content Analyser (RCA) module. The instrument was governed via the Vision v. 2.51 software package, also from Foss NIRSystems. The D-optimal design was developed with the software Modde v. 6.0 (Umetrics). Spectral treatments were applied and multivariate models constructed using The Unscrambler v. 9.8 (Camo Software AS, Oslo, Norway).

Recording of NIR spectra

NIR reflectance spectra for the powdered samples were recorded using a glass cell *ca*. 3 cm in diameter that was placed on the window of the RCA module. Each sample was recorded in triplicate, with rotation between successive recordings, to obtain an average spectrum.

The reflectance spectra for the tablets were obtained by placing each tablet directly on the instrument window and adjusting the shutter aperture to the tablet size. Each sample was recorded in duplicate to obtain an average spectrum.

Construction of calibration and validation sets

Figure 1 depicts the process used to construct the calibration and validation sets. The first step involved calculating the process spectrum, S_P, which incorporates process variability into each tablet:

$$S_P = S_T - S_{lab\ ref}$$

where S_T is the spectrum for a production tablet and $S_{lab\ ref}$ is the reference spectrum (laboratory powder mixture containing the API and excipients at their nominal concentrations). The procedure was applied to 3

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tablets per production batch in order to obtain 3 process spectra (S_P) per batch. By using 3 different batches of the 400 mg tablets and the 600 mg tablets, a total of 9 process spectra for each tablet type (9 S_{P400} and 9 S_{P600}) were obtained. This spectral set constituted the reduced process variability matrix.

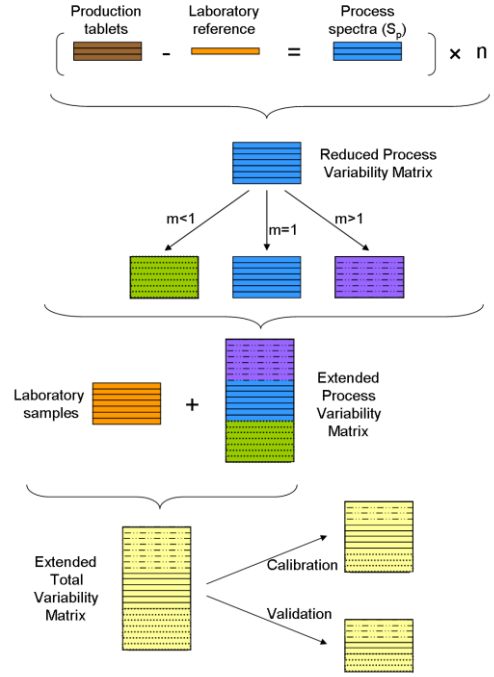

Figure 1. Calculation of the process spectrum.

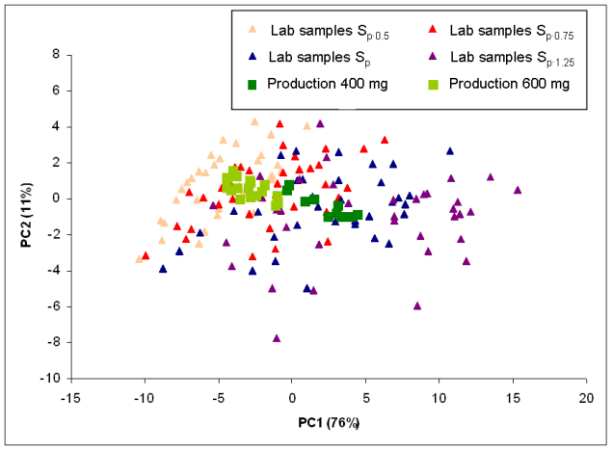

Figure 2. Scatter plot of production and laboratory samples after the addition of the process spectrum.

In order to increase variability in the process spectra, S_{P400} and S_{P600} were multiplied by a factor m equal to 0.5, 0.75 or 1.25. This allowed 18 spectra per m value (9 each for $S_{P400\cdot0.5}$, $S_{P600\cdot0.5}$, $S_{P400\cdot0.75}$, $S_{P600\cdot0.75}$, $S_{P400\cdot1.25}$ and $S_{P600\cdot1.25}$) to be obtained. These 72 spectra and the 18 aforementioned (9 S_{P400} and 9 S_{P600}) constituted the extended process variability matrix and were randomly added to the spectra for the laboratory powder samples:

$$S_{ext\ var} = S_{lab} + S_{P\ ext}$$

where $S_{ext\ var}$ is the spectrum for a powder sample incorporating the variability of the process, S_{lab} that for a laboratory powder sample and $S_{P\ ext}$ a process variability spectrum. The body of $S_{ext\ var}$ constituted the extended total variability matrix, which was split into two subsets: one for constructing the calibration model and the other for validating it.

Reference values

Reference values for the laboratory samples were obtained from the weight of each mixture component. The API contents of the production tablets were determined by HPLC. The average value for each batch was obtained by grinding 20 tablets and treating an aliquot containing the weight of one with 40 mL of mobile phase (a 60:40 v/v acetonitrile/water mixture buffered at pH 3.0), stirring to complete dissolution (~ 30 min) – and making up to volume in a 50 mL flask; an aliquot of the resulting solution was than centrifuged for 5 min and the supernatant injected into the chromatograph for analysis. The API (ibuprofen) was quantified by interpolation of its reading into a calibration curve using isobutylacetophenone as internal standard.

No reference method was available for any of the excipients; therefore, theoretical values which were provided by the pharmaceutical company were used as reference.

Construction of calibration models

Partial least squares (PLS) regression multivariate calibration models were constructed by cross-validation, using the leave-one-out method. A PLS model was constructed over the selected wavelength range for each component of the studied formulation. Various wavelength ranges and spectral treatments (and their combinations) were studied. The best results in all instances were obtained by using a first derivative treatment in combination with an 11 point moving window and subsequent application of standard normal variate (SNV) methodology. Most of the models spanned the entire NIR wavelength range (1100–2500 nm).

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For the silica fumed model it was necessary to apply the Martens' uncertainty test which is based on the jack-knifing principle, to obtain an appropriate wavelength range.

The optimum number of factors was taken to be that leading to the lowest root mean square error (RMSE) and optimally accurate predictions as a result.

Results and Discussion

Constructing an appropriate calibration set requires expanding chemical variability in the samples in order to encompass a wide enough concentration range. Incorporating chemical variability into the calibration set is fairly simple and involves preparing laboratory samples spanning the desired content range for each formulation component. The problem is how to incorporate the physical variability to the calibration set. As noted earlier, the problem can be solved in various ways. In this work, we developed a new, straightforward approach to incorporating the variability of the production process into laboratory samples spanning the required concentration range.

Chemical variability is incorporated into the calibration set using a set of powdered laboratory samples prepared in accordance with a D-optimal design. This allows the desired concentration range for each component to be defined while minimising collinearity between concentration values. Table 1 shows the spectral and concentration correlations for samples prepared following the D-optimal design. It should be noted that spectral correlations between components in the formulation exceeded 0.74 in all cases and was specially high (0.97) for maize starch and cellulose; correlation coefficients between concentrations were highest for the ibuprofen–maize starch couple (–0.85). Although a lower coefficient would have been desirable, one should bear in mind that these two components in combination account for 92% of the formulation content (63.5% API and 28.5% excipient); it is therefore virtually impossible to avoid such a high collinearity in a mixture of the two. On the other hand, the spectral correlation for maize starch and cellulose was quite low (0.37).

Table 1. Correlation coefficients of the pure spectra and the concentrations between the components of the formulation.

Correlation Coefficients		Ibuprofen	Maize starch	Cellulose	Fumed silica	Magnesium stearate
Ibuprofen	Concentration	1	-	-	-	-
	Spectral	1	-	-		-
Maize starch	Concentration	-0.85	1	-	-	-
	Spectral	0.76	1	-	-	-
Cellulose	Concentration	-0.63	0.37	1	-	-
	Spectral	0.78	0.97	1	-	-
Fumed silica	Concentration	-0.72	0.57	0.42	1	-
	Spectral	0.88	0.90	0.87	1	-
Magnesium stearate	Concentration	0.03	-0.30	-0.26	-0.07	1
	Spectral	0.89	0.74	0.77	0.74	1

Incorporating the physical variability of the production process into the calibration process is a more complex task since the treatments undergone by the powdered mixture components during the process are reflected in their NIR spectra in a manner that is rather difficult to predict. The NIR spectrum obtained after each production step gathers the effects of the different treatments applied to the initial mixture.[8] In order to incorporate such effects, we constructed the calibration set in terms of the calculated process variability matrix. Such a matrix will only contain the whole variability of the production process if the laboratory mixture used as reference contains exactly the same API and excipient concentrations as the production tablets; otherwise, the results of the ensuing model will be subject to a systematic error.[8]

Combining the spectral matrix for the laboratory powder samples (which incorporated chemical variability) and the extended process variability matrix (which incorporated physical variability) provided a new matrix containing all sources of variability in the tablet production process. As can be seen from Figure 2 (a scatter plot of the PCA scores for laboratory samples added to the extended variability matrix and

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production samples of the two doses studied), the production samples fell in the sample cluster of the extended total variability matrix. Figure 2, which additionally shows the origin of each type of sample, reveals the way the laboratory samples clustered according to each factor. This allowed assessment of the usefulness of each individual factor. As can be seen, the laboratory samples to which a factor m of 1.25 was applied fell on the right-hand side of the graph, far from the production samples. This led us to exclude them from the calibration set as they were judged redundant in terms of incorporating variability of the production process and would simply complicate the PLS models. On the other hand, the samples to which m = 0.5 was applied fell on the boundary for the cluster of the 600 mg tablets and were judged indispensable for inclusion in the calibration set. The samples to which m = 0.75 was applied were also discarded since using only those with a factor of 0.5 and 1 sufficed to span the whole experimental surface and allowed fairly uncomplicated PLS models to be established.

The approach described above allows the entire variability of the production process to be incorporated via a small number of production samples, thereby substantially reducing experimental work as it avoids the need to record spectra for a large number of samples.

The extended total variability matrix contained mixtures spanning a wide enough concentration range for each component of the formulation to allow a PLS calibration model for each analyte (API and excipients) to be constructed. To this end, the matrix was split into two subsets that were used to construct the calibration models and assess their predictive ability, respectively. Table 2 shows the characteristics of the ensuing models for the API and the four excipients.

Table 2. Figures of merit for PLS models for API and excipients.

	Ibuprofen		**Maize starch**		**Cellulose**		**Fumed silica**		**Magnesium stearate**	
	Cal	Pred	Cal	Pred	Cal	Pred	Cal	Pred	Cal	Pred
Spectral Treatment	1D + SNV		1D + SNV		1D + SNV		1D + SNV		1D + SNV	
Wavelength Range (nm)	1100-2500		1100-2500		1100-2500		1100-2500		1100-2500	
Concentration range (% w/w)	50.8-74.6		18.3-37.7		2.9-8.4		1.2-2.7		0.0-6.9	
PLS factors	3		4		11		10		5	
Concentration Variance (Y) (%)	97.4		97.8		97.1		97.0		99.2	
Offset	2±3	1±3	0.6 ±1.0	1.0 ±2.0	0.2 ±0.2	-0.3 ±0.5	0.1 ±0.1	-0.1 ±0.3	0.01± 0.05	0.03 ±0.07
Slope	0.97 ±0.04	0.98 ±0.04	0.98 ±0.04	0.97 ±0.05	0.97 ±0.05	1.03 ±0.9	0.97 ±0.06	1.0 ±0.2	0.99 ±0.02	0.98 ±0.03
RMSEC/P	0.86	1.00	0.64	1.03	0.24	0.49	0.06	0.12	0.15	0.24

The five models established were used to predict a set of production tablets. A total of 10 tablets from each of 10 different batches of the 400 mg formulation and 18 of the 600 mg formulation were used to record NIR spectra and predict the concentrations for each analyte and tablet. The final results were obtained by averaging the values for the different batches of each formulation. Table 3 lists the predictions and their standard deviations, as well as the average API content as obtained by HPLC and the theoretical reference values for the excipients.

The HPLC technique was used to determine the average API content for each batch and compare it with the NIR result via a paired t-test. The test result for the 400 mg tablets was $t_{calc} = 0.12$ (vs $t_{crit,\ 0.05/2,\ n\ =\ 10} = 2.26$) and that for the 600 mg tablets $t_{calc} = 1.99$ (vs $t_{crit,\ 0.05/2,\ n\ =\ 18} = 2.11$); therefore, t_{calc} was smaller than t_{crit} in both cases and the two methods can be assumed to provide statistically similar results. Because no alternative methods for quantifying the excipients were available, their NIR values were compared with their theoretical contents; in all cases, the predictions of the models were quite similar to the theoretical values and the weighted standard deviation comprised the theoretical value for each excipient (see Table 3).

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Table 3. NIR content uniformity testing.

		400 mg	600 mg
Ibuprofen	NIR value (% w/w)	64.1 (0.8)*	63.5 (1.1)*
	Reference value (% w/w)	64.2 (0.9)**	64.0 (1.0)**
Maize starch	NIR value (% w/w)	28.2 (0.6)*	28.3 (0.9)*
	Theoretical value (% w/w)	28.5	28.5
Cellulose	NIR value (% w/w)	5.3 (0.5)*	5.4 (0.5)*
	Theoretical value (% w/w)	5.0	5.0
Fumed silica	NIR value (% w/w)	2.1 (0.1)*	2.1 (0.3)*
	Theoretical value (% w/w)	2.0	2.0
Magnesium stearate	NIR value (% w/w)	0.9 (0.1)*	0.9 (0.2)*
	Theoretical value (% w/w)	1.0	1.0

*Weighted standard deviation **Standard deviation

Conclusion

Constructing PLS models using the proposed procedure provides clear advantages over the alternative approaches described earlier. The proposed approach allows the construction of accurate quantitation models not only for the API but also for all excipients in a tablet, using a set of samples containing the component concentrations required by a D-optimal design.

Using the extended process to incorporate physical variability in the production process into the calibration set proved an effective strategy. Multiplying the process spectrum by variable factors provided a new experimental domain encompassing production samples; this resulted in improved predictive ability in the ensuing models, as reflected in the low RMSEP values obtained (< 1.0%) and in the results of a paired t-test applied to the API predictions.

The concentrations of API and excipients used to construct the PLS models were established by weighing with an analytical balance; this dispenses with the need for an alternative method to determine the reference API and excipient concentrations. The proposed approach therefore releases NIR spectroscopy from the need for a reference method to construct quantitation models for this type of sample and improves accuracy and precision in the results.

The simplicity of the approach, and especially the quality of its results, clearly makes it an effective alternative to conventional methods for constructing NIR calibrations for pharmaceutical analysis.

Acknowledgements

The authors are grateful to Spain's Ministry of Science and Innovation (MICINN) for funding this research within the framework of Project CTQ2009-08312.

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