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Real-time determination of critical quality attributes using near-infrared spectroscopy: A contribution for Process Analytical Technology (PAT)

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ABSTRACT

Process Analytical Technology (PAT) is playing a central role in current regulations on pharmaceutical production processes. Proper understanding of all operations and variables connecting the raw materials to end products is one of the keys to ensuring quality of the products and continuous improvement in their production. Near infrared spectroscopy (NIRS) has been successfully used to develop faster and non-invasive quantitative methods for real-time predicting critical quality attributes (CQA) of pharmaceutical granulates (API content, pH, moisture, flowability, angle of repose and particle size). NIR spectra have been acquired from the bin blender after granulation process in a non-classified area without the need of sample withdrawal. The methodology used for data acquisition, calibration modelling and method application in this context is relatively inexpensive and can be easily implemented by most pharmaceutical laboratories. For this purpose, Partial Least-Squares (PLS) algorithm was used to calculate multivariate calibration models, that provided acceptable Root Mean Square Error of Predictions (RMSEP) values ($RMSEP_{API}$=1.0 mg/g; $RMSEP_{pH}$=0.1; $RMSEP_{Moisture}$=0.1%; $RMSEP_{Flowability}$=0.6 g/s; $RMSEP_{Angle\ of\ repose}$=1.7° and $RMSEP_{Particle\ size}$=2.5%) that allowed the application for routine analyses of production batches. The proposed method affords quality assessment of end products and the determination of important parameters with a view to understanding production processes used by the pharmaceutical industry. As shown here, the NIRS technique is a highly suitable tool for Process Analytical Technologies.

1. Introduction

Pharmaceutical production processes comprise a number of steps that involve a series of operations requiring validation and adherence to strict Standard Operating Procedures (SOPs). Quality Assurance, which encompasses the decisions leading to the successful completion of each step in a process, relies on off-line testing to assess the quality of a product at the end of each step, as well as that of the end product. This takes a long time that is further increased by the manufacturing cycle time. In addition, because this approach considers neither risk assessment nor risk management, it does not ensure the complete absence of quality defects in the product. This problem was addressed by Process Analytical Technology (PAT) initiative of the United States Food and Drug Administration (US-FDA) [1] aimed at fostering the development of more technically and scientifically rigorous production processes by the pharmaceutical industry [2].

The PAT approach, which relies on scientific knowledge and risk analysis, affords the design and development of efficient, continuously controlled processes. In this way, it ensures a preset level of quality at the end of the manufacturing process. PAT can therefore be regarded as a joint venture of analytical chemical science and pharmaceutical technology [3–5].

Near infrared spectroscopy (NIRS) is one of the most flexible vibrational spectroscopic techniques for the analysis of pharmaceutical products and also one of the most useful tools for the industrial implementation of PAT on account of its affording at-line, in-line and on-line measurements by virtue of its ability to measure a number of physical and chemical properties of samples. Some of the better known uses of NIRS in the production of solid pharmaceutical forms include chemical raw material identification [6], blend uniformity assessment [7–10], granulation monitoring [11], roller compaction monitoring [12], drying end-point determination [13] and coating end-point and uniformity determinations [14].

Abbreviations: SOPs, Standard Operating Procedures; PAT, Process Analytical Technology; US-FDA, United States Food and Drug Administration; NIRS, near infrared spectroscopy; HPLC, high-performance liquid chromatography; ISO, International Organization for Standardization; CQA, critical quality attribute; API, active pharmaceutical ingredient; PLS, Partial Least Squares; PCA, Principal Component Analysis; SNV, Standard Normal Variate; RMSE, Root Mean Square Error; RMSEC/P, Root Mean Square Error of Calibration/Prediction; S_p, process spectrum

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Using NIRS in PAT projects has a number of advantages including its non-destructive nature and its ability to provide immediate results, which affords real-time analysis. Near infrared spectra are influenced by some physical properties of the samples, but the effects can be suppressed or minimised by using an appropriate spectral pretreatment; also, the results must be calibrated against a reference technique such as HPLC, but a recent method allows the easy construction of NIR calibration models for quantifying the API and excipients in a formulation without the need for a reference method [15].

In this work, we developed a method for the non-invasive determination of the critical quality attributes (CQAs) of a pharmaceutical granulate with a view to reducing its manufacturing time and obtaining a better knowledge of the influence of some variables of the production process. At present, the determination of CQAs for a granulate involves withdrawing a sample from the bin blender in an ISO 8 classified area and submitting it to the laboratory for chemical analysis. In addition to the need for an ISO 8 area and its staffing, this entails taking some health cautions, which delays the sampling process. Also, the conventional methodologies used for this purpose are time-consuming, which additionally delays the obtainment of quality information for timely decision-making (specifically, whether a given production batch should be released for the next step of the process, which is usually packaging).

This PAT methodology was used to determine the target CQAs (API content, pH and moisture content as primary attributes) from NIR spectra acquired in the bin blender after the granulation process in a non-classified area without the need of sample withdrawal. Also, it is intended to evaluate the determination of other physical variables, such as flow-related properties (flowability and angle of repose) and particle size-related parameters (<125, 125–250 and >250 μm fractions) that can eventually influence the quality of the end product.

2. Materials and methods

2.1. Pharmaceutical formulation

The formulation used was a solid form commercially available in 2 g bags and containing an amount of active pharmaceutical ingredient (API) of 50 mg per gram of product (5 wt%), sucrose as major excipient (90 wt%), citric acid as minor excipient (1.5 wt%), macrogol 400 (0.4 wt%), maltodextrin (0.8 wt%) and orange flavouring (2.1 wt%). The API (Nimesulide) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antithermal action.

2.2. Calibration samples used for PLS models

A total of eight multivariate calibration models were constructed by using the Partial Least-Squares Algorithm (PLS1) for the following CQAs: (1) API content; (2) pH; (3) moisture content; flow-related properties (4) flowability and (5) angle of repose; and particle size (6) >125 μm, (7) 125–250 μm and (8) >250 μm.

The models used to determine the API and pH were constructed from the spectra of 55 powder mixtures of the formulation ingredients prepared in the laboratory by weighing on an analytical balance and mixed in a Turbula solid blender. The composition for the sample set was established by using a D-optimal design, modifying the concentrations of the five components of the formulation (API, sucrose, citric acid, maltodextrine and orange flavouring) in order to minimise correlation between concentrations. The concentration range used and the matrix of correlations between components in the mixtures are shown in Table 1. As can be seen, correlation was minimal except for the API and sucrose, the high concentrations of which (95% of the mixture in combination) precluded lowering the correlation level. Sample set was split in two subsets, for calibration and validation purposes.

The models for moisture, flow-related properties and particle-size related properties were constructed by using the spectra for samples from 12 different production batches.

For the moisture model, a subset of samples was dried in a laboratory-drying oven and another subset was wetted in a wet chamber by placing it next to a vessel with water in a closed enclosure in order to expand the calibration range with respect to the nominal value. Sufficient amount of sample to determine moisture by the reference method and recording the spectrum was extracted every hour to cover a suitable range of moisture.

The granulate samples used to construct the models for particle size and flow properties were sieved to retain three different size fractions which were mixed in appropriate proportions in order to expand the calibration range for flowability.

2.3. Calibration modelling with the S_p method

The API and pH calibration models were constructed by using the method proposed by Blanco and Peguero [15], except that data were obtained from SNV-processed rather than direct absorbance data. Briefly, the method involves calculating the process spectrum, S_p, which should contain the variability of the production process (Eq. 1). These changes during production process are reflected in the spectra as a spectral offset, scaling, etc. as a result of the granulation step. Consequently S_p spectrum could be different for different granulation behaviour:

$$S_p = S_t - S_{tab\_ref} \quad (1)$$

where S_t is the spectrum for a production sample and S_{tab_ref} the reference spectrum, corresponding to a powder laboratory mixture containing the API and excipients at their nominal concentrations (see Fig. 1).

2.4. Granulation

Granulation was done in a GLATT WSG300 fluid bed system (FBS) where the raw materials (API and excipient) were introduced

Table 1
Correlation coefficients between concentrations of the powder samples components.

Compounds	Correlation coefficients					Concentrations	
	API	Sucrose	Citric acid	Maltodextrin	Orange flavouring	Range (mg/g)	Nominal approx. (mg/g)[a]
API	1					34–66	50
Sucrose	−0.97	1				890–921	900
Citric acid	0.16	−0.24	1			12–18	15
Maltodextrin	−0.17	0.11	−0.14	1		5–9	7
Orange flavouring	−0.28	0.15	−0.42	−0.03	1	17–28	21

[a] The missing 4 mg/g of macrogol to complete the 100% of formulation is added as solution during granulation process.

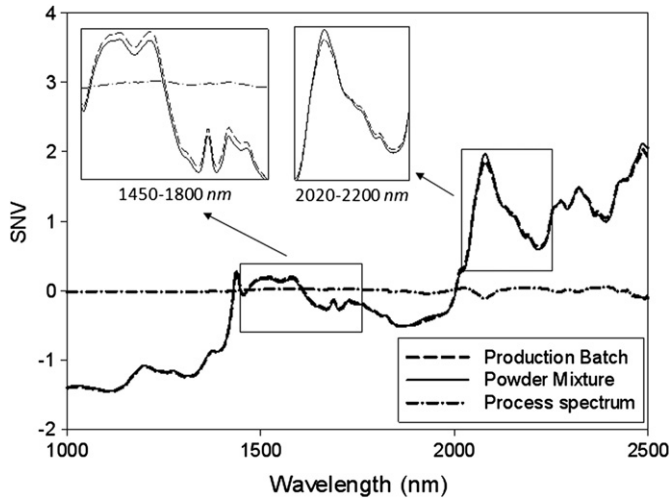

Fig. 1. SNV spectra for a laboratory powder mixture, a granule production sample (both containing the same concentration of each component) and a process spectrum.

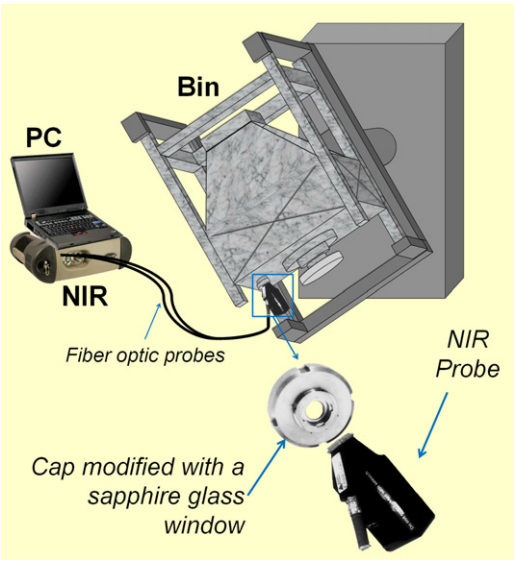

Fig. 2. Measuring schema for non-invasive NIR spectra recording in the bin.

by aspiration and an ascending flow or hot air continuously agitated the load in order to ensure uniform blending prior to granulation. After the binder solution (an aqueous solution of macrogol 400, sucrose and maltodextrin) was added and the granulate formed, the hot air flow was continued until the required moisture level was reached. The overall process time was about 255 min. The granulate was transferred to stainless steel bin blenders for mixing with an extra-granular excipient and the flavouring agent which was not added during granulation in order to avoid its loss through drying. Once the mixing process was finished, a sample was withdrawn to determine the API by HPLC and the result was used to ascertain whether the batch concerned was released for subjection to the next steps of the manufacturing process (dosage and packaging) or held in the bin blender for further agitation to complete uniformity. The analytical determination of the API can take several hours, which, in addition to withdrawing samples in a classified zone, considerably delays the manufacturing process.

2.5. Reference methods

All methods used to obtain the reference values were previously validated by Laboratorios Menarini and are routinely used by the control laboratory for the analysis of production batches. The API (Nimesulide) was quantified on an HPLC instrument from Agilent Technologies (Santa Clara, CA, USA) furnished with a Lichrospher 100 RP-18 column. The mobile phase was a 40:60 (v/v) mixture of 0.03 M acetic acid and methanol, and the spectral wavelength of 300 nm. pH measurements were directly made in 2% (w/v) solutions of the granulate, using a model 691 pH-meter from Metrohm AG (Herisau, Switzerland). Moisture contents were determined by using the loss-on-drying method on a LJ16 Moisture Analyzer Balance from Mettler Toledo Intnal., Inc. (Greifensee, Switzerland), each sample being heated at 90 °C to constant weight for 5 min.

Particle size was determined by sieving various granulate batches through a Prufsieb Jel 200 sieve shaker (Hosokawa, Augsburg, Germany) in order to obtain three particle fractions: >125, 125–250 and >250 μm.

Flowability and angle of repose were determined with a Powder Characterisation Instrument GmbH PTG-2 from Pharma Test AG (Munich, Germany).

2.6. Near infrared equipment

Near infrared spectra were recorded in the bin blender, using a Portable LabSpec Pro 2500 NIR spectrophotometer from ASD, Inc. (Boulder, CO, USA). Measurements were made in the reflectance mode, using a 3 mm thick sapphire window 2 cm in diameter that was inserted into a bin blender cap previously adapted for insertion of a probe that was connected to the spectrophotometer via a 3 m long optical fibre. The experimental set-up used is depicted in Fig. 2.

The spectra for the laboratory calibration samples were recorded by using an identical probe. An appropriate amount of sample was placed on the bin blender cap in such a way as to completely cover the sapphire window.

All NIR spectra were acquired with the aid of the software Indico Pro 5.2, also from ASD, Inc. Each spectrum was the average of 32 scans spanning the range 1000–2500 nm at 1 nm intervals.

2.7. Spectral processing

The spectral treatments used included Standard Normal Variate (SNV) transformation, first and second Savitzky–Golay derivatives with a moving window of 11–21 points, and combinations of SNV with derivatives and Principal Component Analysis (PCA). Models were constructed by full cross-validation and validated against an external data set. PLS models were developed with the software The Unscrambler v. 9.8, from CAMO (Trondheim, Norway). Unscrambler On-line v. 2.2, also from CAMO, was used to apply the ensuing models to the manufacturing process in order to predict physical and chemical CQAs. The quality of the models was assessed in terms of the Root Mean Square Error (RMSE):

$$\mathrm{RMSE} = \sqrt{\frac{\Sigma_{i=1}^{n}(y_i^{pred} - y_i^{ref})^2}{n}} \quad (2)$$

where n is the number of samples, y^{ref} the reference value and y^{pred} the NIR predicted value.

3. Results and discussion

The sequence of steps during the granulation process studied, which involves six steps (steps 1 and 2 are not described here as they have been examined in another study), is illustrated in Fig. 3.

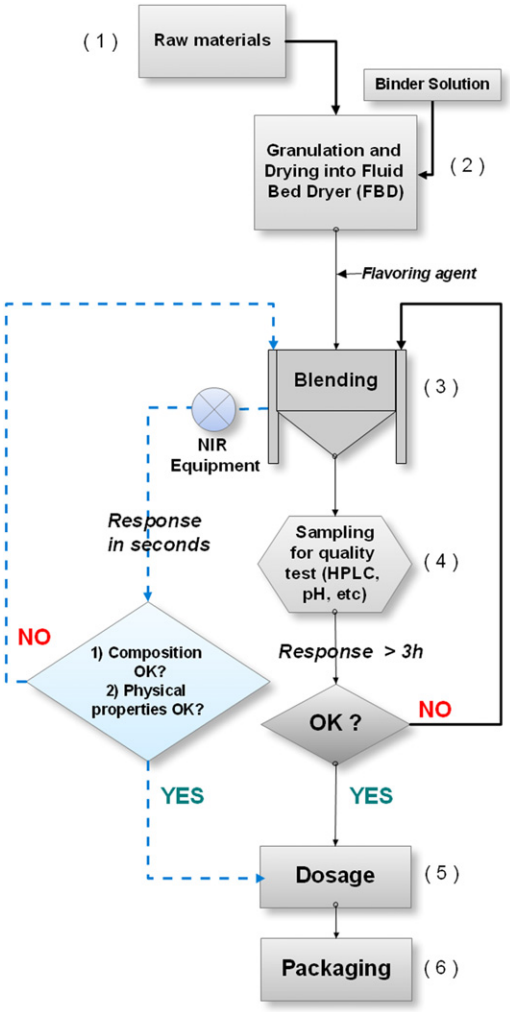

Fig. 3. Steps for current granulation manufacturing process and for the new NIR method implementation in routine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As can be seen, the scheme shows the conventional (step 4) and proposed strategy (in dotted blue lines) for quality assessment of blending step, the most interesting step for our purposes, which can be substantially expedited by using the proposed strategy to real-time to ensure that the blend is uniform. In a routine process, the average time elapsed between sample collection (step 4) for analysis to delivery of the results is typically 6 h (in a quality control laboratory not exclusively dedicated) and depends on various external factors such as the availability of staff and an ISO 8 classified area for sampling, as well as the time during which batches are stocked unprocessed. Obtaining accurate analytical results in real-time in this step allows one to assure that the product is suitable for subjection to the following last steps of the process (dosage and packaging) or else be corrected as required prior to release (with extra blending time for example). The physical and chemical information provided by PLS models for this step of the process is much wealthier than that obtained with the conventional methodology and takes only a few seconds to acquire.

3.1. Development of the calibration model

The samples to be included in the calibration and validation sets for constructing our multivariate calibration models were selected by using a scatter plot for the first two scores of a PCA [16] to ensure that the calibration samples would encompass the whole variability in the prediction samples; if the available samples failed to fulfil this condition, then the sample set had to be expanded with selected or properly prepared samples. Determining the API content and pH in the bin blender required constructing PLS models from the spectra for laboratory powder samples, which were combined with the process spectrum (S_p) to incorporate process variability in the calibration and prediction sets. For example, whether the sample set combined with S_p contained the process variability was assessed by PCA of the calibration set for the API and projecting the scores for the external validation samples. As can be seen from Fig. 4a, the first two principal components (PCs) explained 87% of the total variance. PC1 (60%) was associated to processes variability including the spectrum process (S_p), separating the samples into two clusters with similar S_p. PC2 and PC3 (27% and 7% respectively) were associated to changes in the concentration of the API. As can be seen in the example of Fig. 4a, the calibration samples encompassed the prediction samples, showing that the samples are suitable for constructing the PLS model to quantify API. Actually, these NIR methods will be used in the bin blender, consequently it is necessary to ensure that the calibration samples collect variability of production batches. For example, whether the sample set combined with S_p contained the process variability was assessed by PCA of the calibration set for the API and the scores were projected for the production batches. As can be seen from Fig. 4b, the first two principal components explained 87% of the total variance; also, the production samples were included in the set of laboratory samples combined with the process spectrum, which confirmed that the expanded calibration set encompassed the variability in the production samples and was hence appropriate for constructing the models. The narrow concentration range spanned by the production samples ($\pm$5% around the nominal value) is consistent with their clustering in the middle of the graph; by contrast, the laboratory samples spanned a wider concentration range and their scores were more scattered along the PC2 axis.

Fig. 4c shows the first two PCA loadings of the calibration samples. If these loadings are compared with a production batch and API spectra (Fig. 4e) we can clearly see that the first loading shows characteristic bands of production batch spectrum, especially in the range 1400–1600 and 2000–2100 nm (characteristic bands of sucrose, see Fig. 4f). In Fig. 4c it can be observed that the second loading is related to API (Fig. 4e) mainly in the bands around 1500, 1650, 1900, 2100–2300 nm.

Table 2 shows the figures of merit of the PLS models for primary CQAs. The three models required three PLS factors each to explain 99% of the variance with virtually the entire wavelength range—the zone from 2300 to 2500 nm was excluded owing to the high noise in the derivative spectra. As an example, Fig. 4d shows the three loadings of PLS model for API which explained 99.4% of variance. As can be seen, the first two loading (86.8% and 12.1%) are closely related to the API, especially in the bands around 1150, from 1500 to 1700 and 2100–2300 nm (Fig. 4f). The third loading (0.5%) is related principally to sucrose, especially in the bands around 1300–1450 and 1900–2100 nm (Fig. 4f). With respect to the PLS model for pH, Fig. 5a shows the first two loadings explaining 95.5% of the variance. As can be seen, the first loading is closely related to the citric acid (Fig. 5b), especially in the bands 1420, 1650–1750, and 2025–2090 nm. The second loading seems to be related to certain bands of API (Fig. 5b), mainly around 1675, 1834, and 2150–2210 nm. It is important to clarify that calibration samples for pH contained principally variable amounts of citric acid, but as we have seen it is not the only component related to pH, since the API also contributes to pH changes, consequently pH depends on the

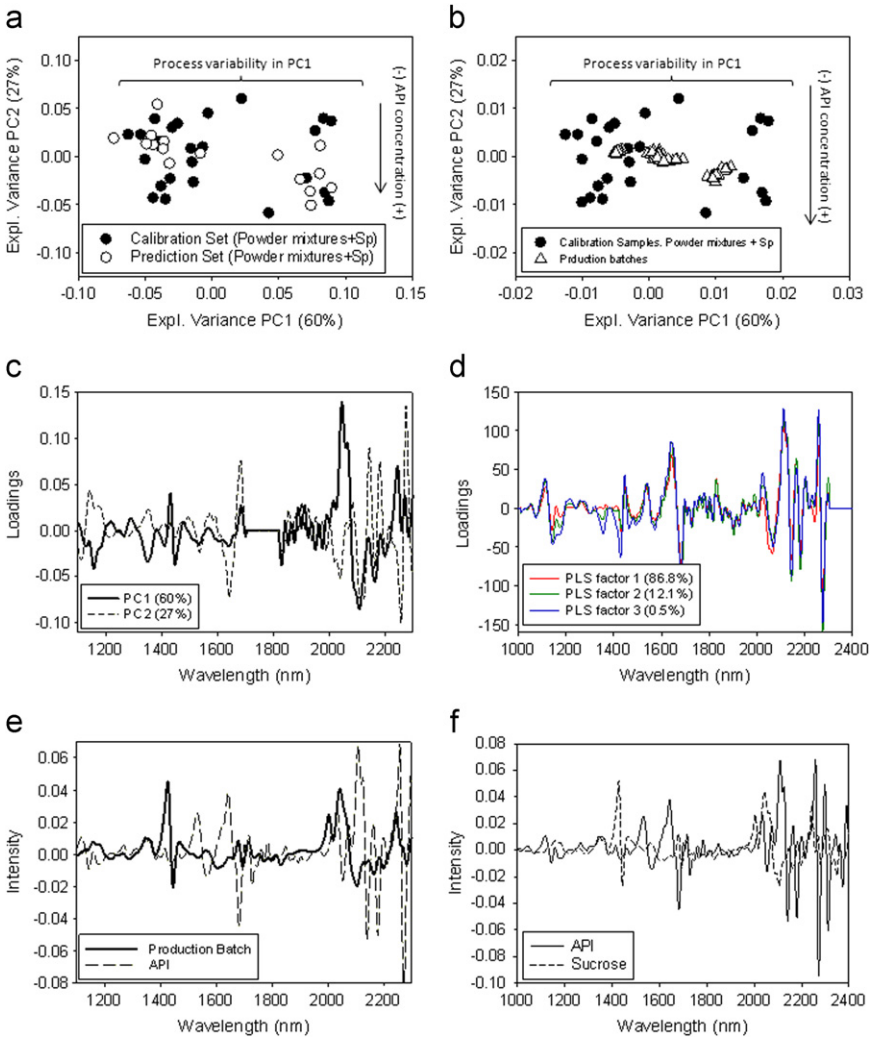

Fig. 4. Graphic details of multivariate analysis of samples set to develop NIR method to quantify API. (a) PCA: projection of validation samples in scatter plot of scores from PCA of calibration samples, (b) PCA: scatter plots of production samples and laboratory calibration samples (powder mixtures + spectrum process), (c) PCA: plot of first two loadings explaining 87% of variance. (d) PLS: three first loadings of PLS model for API, (e) PCA: production batch and API spectra, and (f) PLS: API and sucrose (major excipient) spectra. Spectral mode used in all case was SNV + 1st derivative in 1100–2300 nm range.

Table 2
Figures of merit of the PLS models constructed for primary CQAs.

Set	Characteristics	API (mg/g)	pH	Moisture (%)
Calibration Set	No. of samples	25	39	23
	Spectral pre-treatment	SNV + 1st derivative	SNV + 2nd derivative	1st derivative
	Wavelength range (nm)	1000–2300	1000–2260	1300–1500 1850–2050
	Calibration range[a]	34.1–66.2	2.9–3.3	0.10–1.21
	Nominal values (NOC)[a]	50.0 ± 2.5	3.0 ± 0.5	< 0.5
	Number of PLS factors	3	3	3
	Explained variance (Y) (%)	99.4	99.1	97.8
	Regression Y^{ref} vs. Y^{NIR}			
	Slope $\pm$ $CI_{\alpha=0.05}$	0.99 ± 0.03	0.99 ± 0.03	0.98 ± 0.07
	Offset $\pm$ $CI_{\alpha=0.05}$	0.31 ± 1.72	0.03 ± 0.10	0.01 ± 0.03
	RMSEC[a]	0.68	0.01	0.04
Prediction set	Prediction range[a]	40.8–56.2	3.03–3.10	0.11–0.70
	No. of samples	19	12	9
	RMSEP[a]	1.0	0.1	0.1

NOC = Normal Operating Conditions.
CI = Confidence interval ($\alpha = 0.05$).
RMSEC/P = Root Mean Square Error of Calibration/Prediction.
[a] Results are expressed in their respective units.

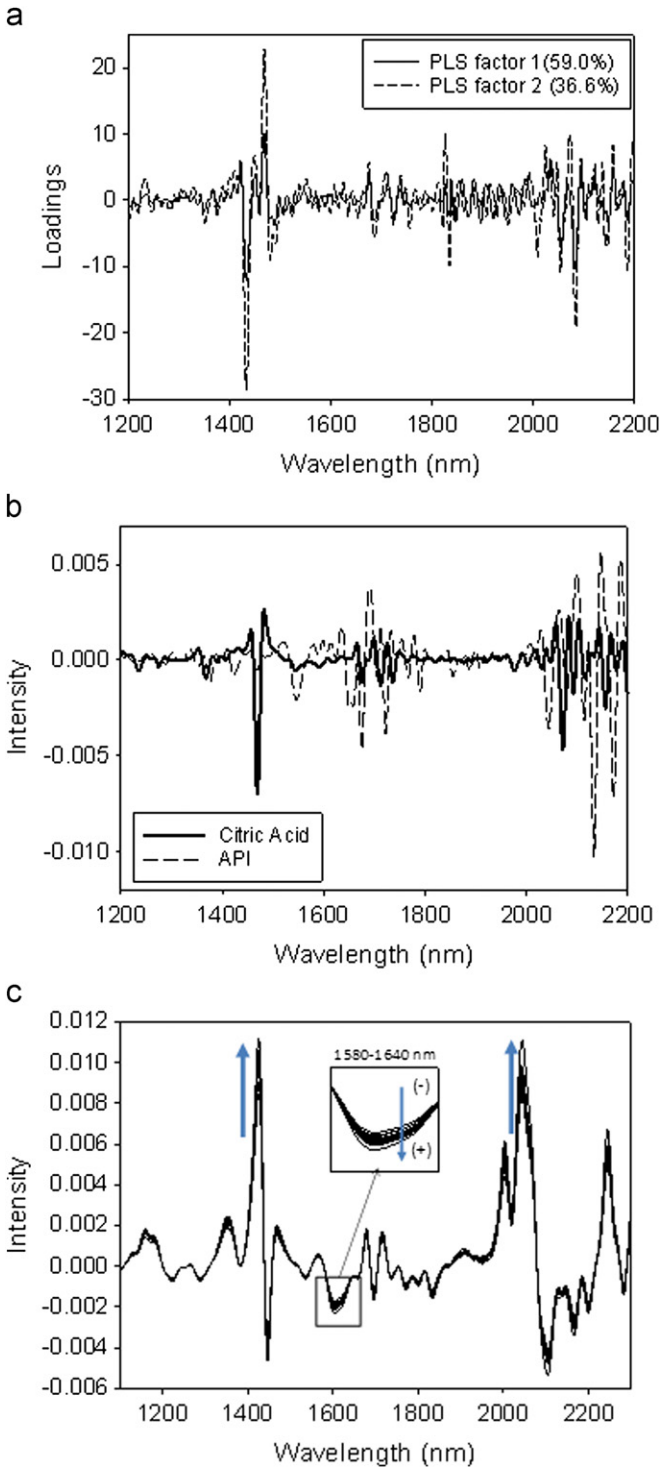

Fig. 5. Details of PLS models for pH and flowability. (a) Two first loadings of PLS model for pH, (b) SNV+2nd derivative spectra of citric acid and (c) 1st derivative spectra of calibration set used to construct PLS flowability model.

proportion of these two substances. The model for moisture spanned the wavelength regions containing the typical bands for water (see Table 2). All models were validated against an external set of prediction samples not used for calibration and all exhibited acceptable prediction errors.

Table 3 shows the figures of merit of the PLS models for secondary CQAs. These models required an increased number to explain 99% of the variance relative to those for primary CQAs. Because the determination of physical parameters by NIRS cannot rely on a characteristic absorption band, the models spanned the whole wavelength range. Although they required the same number of PLS factors, the models for flowability and angle of repose provided smaller errors than those for particle size. As an example, Fig. 5c shows the spectra of the calibration set used to construct the PLS model for flowability, as can be seen there is a variation along the spectrum, however there are bands related to changes in flowability, especially around 1400, 1580–1640 and 2000 nm (by using Jack-knife criterion to evaluate significance of regression coefficients, we found two important regions at 1380–1613 and 1980–2071 nm). As can be seen, the bands of water are situated between these ranges, as a consequence we can affirm that the moisture content has an effect on the flowability and consequently on the angle of repose. Similar results were found by Otsuka [17] and Sarraguca et al. [18] in studies about physical properties by using NIRS. By the use of Jack-knife criterion, significant ranges for angle of repose PLS loadings were found at 1126–1445 and 1880–2332 nm. On the other hand, PLS loadings for particle size model (plot not shown) were similar to the mean calibration spectrum, because when particle size increases a positive baseline displacement is observed on the spectra.

The samples used to expand the flowability and particle size range were mixtures containing variable proportions of granulate in different particle sizes. These samples were used to determine the correlation coefficient between the two properties. As expected – both parameters are measures of the same property – flowability and the angle of repose were highly correlated ($R=-0.96$); also, both were highly correlated with the proportion of fine particles (<125 μm, $R=-0.93$) (see Table 4).

The proposed NIR method to quantify API was validated for use in the routine analysis by using the model obtained. Validation was based on the guidelines of the International Conference on Harmonisation (ICH) [19] and included selectivity, linearity, accuracy and robustness.

The selectivity of an NIR method is established by the use of spectral libraries, which allow the accurate identification of the pharmaceutical preparation as a combination of API and excipients. The identification criterion used was the correlation coefficient (CorrCoef), with a threshold of 0.98. Spectral pre-treatment used was second-derivative mode over the wavelength range of 1100–2200 nm. All production batches were positively identified (CorrCoef >0.98) and every pure component in the formulation was accurately discriminated at an identification level above the threshold (CorrCoef <0.98). Table 5 shows the identification values.

The application of proposed method was found to meet all the requirements in the validation guideline, which confirms its suitability for use as a routine analytical method in the pharmaceutical industry. Table 5 shows the most salient results for the validation procedure of the application of NIR method for API in the pharmaceutical process. The application in routine for the rest of the NIR methods (pH, moisture, flowability, angle of repose and particle sizes) is in the process of validation. This procedure took some time because it is necessary to sieve the samples from different batches and then analyse the fractions using the reference. However, the robustness values of all NIR methods were evaluated in several production batches such as described below.

3.2. Validation of robustness for proposed methods. Non-invasive determination of CQAs in the bin blender

The robustness for above-described PLS models were validated by using them to determine the CQAs for fifty industrial batches and assess their robustness in routine analyses. To this end, a total of six NIR spectra per batch were recorded as described in Section 2.6 and averaged. The results thus obtained were compared with

Table 3
Figures of merit of the PLS models constructed for secondary CQAs.

Set	Characteristics	Flow properties		Particle size parameters		
		Flowability (g/s)	Angle of repose (deg.)	< 125 μm (%)	125–250 μm (%)	> 250 μm (%)
Calibration set	No. of samples	17	15	10	9	9
	Spectral pre-treatment	1st Derivative	SNV + 1st derivative	1st Derivative	SNV	SNV
	Wavelength range (nm)	1100–2330	1000–2400	1000–2500	1000–2400	1000–2400
	Calibration range[a]	4.4–9.3	30.3–36.3	16.6–25.0	35.5–43.5	37.1–48.0
	Number of PLS factors	5	6	5	5	6
	Explained variance (Y) (%)	97.9	99.6	99.9	99.4	99.3
	Regression Y^{ref} vs. Y^{NIR}					
	Slope $\pm$ $CI_{\alpha=0.05}$	0.98 ± 0.08	1.00 ± 0.04	1.00 ± 0.02	0.99 ± 0.07	0.99 ± 0.07
	Offset $\pm$ $CI_{\alpha=0.05}$	0.15 ± 0.56	0.14 ± 1.28	0.01 ± 0.31	0.22 ± 2.60	0.26 ± 2.99
	RMSEC[a]	0.18	0.11	0.04	0.18	0.24
Prediction set	No. of samples	23	29	4	6	5
	Prediction range[a]	5.9–7.4	30.8–35.1	18.4–19.7	37.9–42.0	40.9–46.0
	RMSEP[a]	0.6	1.7	2.5	2.4	2.5

CI = Confidence interval ($\alpha = 0.05$).
RMSEC/P = Root Mean Square Error of Calibration/Prediction.
[a] Results are expressed in their respective units.

Table 4
Correlation coefficients of the physical parameters.

	< 125 μm	125–250 μm	> 250 μm	Flowability (g/s)	Angle of repose (deg.)
< 125 μm	1				
125–250 μm	−0.50	1			
> 250 μm	−0.50	−0.50	1		
Flowability (g/s)	**−0.93**	0.56	0.37	1	
Angle of repose (deg.)	**0.95**	−0.59	−0.36	**−0.96**	1

Table 5
Validation parameters for the API quantitation in pharmaceutical process by using the proposed NIR method.

	Compound	Coefficient correlation
Selectivity	Production batch	0.998
	Powder sucrose	0.978
	Crystal sucrose	0.955
	Maltodextrin	0.239
	Orange aroma	0.200
	Citric acid	0.064
	Nimesulide	−0.061
Threshold 0.98 (positive identification: id results > 0.98)		
	Parameter	Result
Linearity	n	9
	Concentration range (mg/g)	36.3–60.7
	Intercept	2.23 ± 2.32
	Slope	0.96 ± 0.05
	R	0.991
Accuracy	n	
	Average difference (mg/g)	9
	S.D.	0.55
	t_{exp}	1.42
	t_{crit}	1.17
Repeatability	Replicates	2.31
	Mean NIR (mg/g)	
	CV (%)	6
Robustness	n	50
	t_{exp}	1.50
	t_{crit}	2.01

Table 6
Non-invasive NIR method robustness for critical quality attributes (CQAs).

	Parameter	REF_{Mean}	NIR_{Mean}	RES_{Mean}	RMSEP	t_{obs}
Primary CQAs	API (mg/g)	49.83	50.44	0.61	1.34	1.50
	pH	3.09	3.08	−0.01	0.03	0.60
	Moisture (%)	0.21	0.24	0.03	0.05	1.70
Secondary CQAs	Flowability (g/s)	6.78	6.82	0.04	0.57	0.31
	Angle of repose (deg.)	33.36	33.04	−0.32	0.94	0.99
	< 125 μm (%)	19.18	19.85	0.67	1.46	1.70
	125–250 μm (%)	38.51	38.39	−0.12	1.22	0.27
	> 250 μm (%)	42.32	41.84	−0.48	1.39	1.00

$t_{crit} = 2.01$ ($\alpha = 0.05$ and 49 degrees of freedom). t_{obs} lesser than t_{crit} indicates two means are not statistically different.
REF_{Mean} refers to mean of the reference values and NIR_{Mean} refers to mean of the predicted values by NIR model for 50 production batches.
RES_{Mean} refers to mean of the residuals values and RMSEP refers to the root mean square error of prediction.

the reference values provided by the routine analytical method applied to each batch, using a paired t-test at the 95% confidence level. As can be seen from Table 6, there were no statistical differences between the mean NIR predicted values and the reference values ($t_{obs} < t_{crit}$). This confirms that the models provided accurate results from non-invasive measurements made in the bin blender.

These results warrant using PLS models in routine analyses with a view to assessing their long-term robustness by application to an increased number of batches. The most salient feature of this new NIR methodology is that it affords the quantitation of a greater number of both physical and chemical properties in a product from a single NIR spectrum, thereby enabling routine determinations which were formerly done on an individual, anecdotal basis.

4. Conclusions

The proposed experimental design, which requires no alteration of the operational procedure, allows the non-invasive and real-time determination of critical quality attributes (CQAs) for pharmaceutical granulates. Our methodology provides a deeper knowledge of the product as it allows the whole physico-chemical information it contains to be extracted in real time (a few seconds) and with no added effort. The information thus obtained can be used to expedite decisions, which formerly required hours, even in the absence of a comprehensive knowledge of the applicable quality specifications. The proposed approach facilitates quantitative assessment of granulation and blending uniformity. Also, the eight PLS models used allow the most salient properties of the granulate to be determined in real time with measurement in a non-classified area. The operational simplicity of method makes it suitable to be executed by non-specialists (i.e, an operator of production). Consequently, NIR spectroscopy is a useful tool for Process Analytical Technology (PAT).

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