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Near-infrared determination of active substance content in intact low-dosage tablets

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Abstract

Near-infrared (NIR) spectroscopy can be applied to determine the active substance content of tablets. Its great advantage lies in the minimal sample preparation required, which helps to reduce the potential for error. The aim of this study is to show the feasibility of this method on low-dosage tablets. The influence of various spectral pretreatments [standard normal variate (SNV), multiplicative scatter correction (MSC), second derivative (D2), orthogonal signal correction (OSC), separately and combined] and regression methods on prediction error are compared. Partial least square (PLS) regression provided better prediction than principal component regression (PCR). SNV was applied to the first data set and SNV and a second derivative to the second set to maximise model accuracy for quantifying the active substance of intact pharmaceutical products using diffuse reflectance NIR. The models yielded standard errors of prediction (SEP) of 0.1768 and 0.0682 mg for the two products. The experiments were conducted with two low-dosage pharmaceutical forms and results of NIR predictions were comparable to currently approved methods. Diffuse reflectance NIR has the potential to become a reliable and robust quality control method for determining active tablet content.

Keywords: Near-infrared spectroscopy; Active content; Intact tablets; Content uniformity; Pretreatment; Regression

1. Introduction

Since Norris and Butler first used near-infrared (NIR) spectroscopy in the 1960s to analyse agricultural samples [1,2], applications have expanded exponentially, due mainly to advances in computational power, multivariate statistics and also to information contained in the spectral range [3]. NIR is now widely used in the pharmaceutical industry to identify raw materials [4], select polymorphs [5,6], and quantify residual moisture in lyophilised proteins in glass vials [7] or in granulate [8]. More recently, it has been employed to determine the site of production of proprietary products [9]. NIR microscopy, increasingly used to collect process-related information [10], has become a key technique in process analytical technology (PAT) [11].

To date NIR has not been widely employed for determining tablet active substance content, except for tablets with either a very high active content or the active as the main component [4,12–14], few studies report the use of NIR for the quantification of low tablets [15,16]. Its overriding advantage is that sample preparation is minimal compared to the time-consuming preparation, solvent constraints and sample destruction typically required by classical analytical methods. In 1990, Corti et al. [17] described the use of reflectance NIR to quantify tablet active content (<0.25 mg), but a chloroform extraction step was required. Quantitative tablet analysis can be done either by transmittance or reflectance [18–22]. Transmittance NIR was used by Eustaquio et al. [23] to quantify paracetamol in intact tablets, where the active substance accounted for 84% of the mass, but also by Broad et al. [24] to quantify tablet steroid content through a wide range of mass concentrations (2.94–17.64%, w/w). Transmittance NIR thus appears

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a powerful tool for determining the active substance content of intact tablets.

The aim of the present study was to show that diffuse reflectance NIR, which is easier to transfer on-line, can achieve accuracy on intact uncoated tablets, even those with low active content. In this objective, several regression methods after applying selected pretreatments separately or in combination on the NIR spectra are compared in order to optimize the model in comparison with the results given by wet analyses. We applied the same optimisation to two pharmaceutical products with lower active substance content, as a proportion of total tablet mass, than in earlier studies [4,12–14] (3 and 1.18% (w/w), respectively), to show that quantitative models can be built on such low-dosage samples, a part of the validation according to the EMEA guideline [25] is down to show the feasibility.

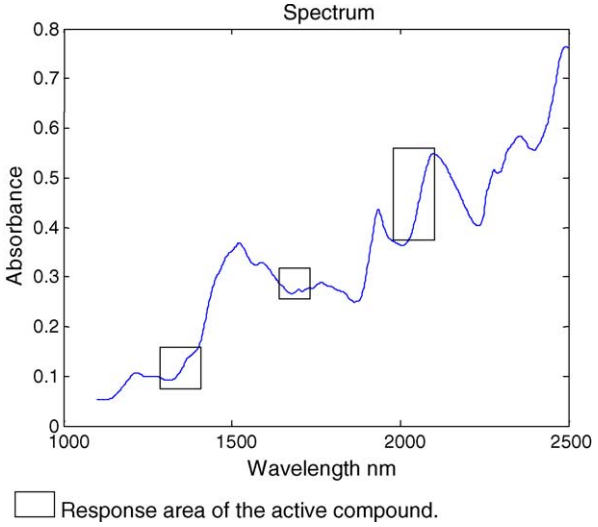

Fig. 1. Raw spectrum of product A.

2. Materials and methods

2.1. Samples and sample preparation

Samples of two products, A and B, were produced by Roche Pharmaceuticals, Basle. The active are bromazepam and clonazepam, respectively, for products A and B. Both tablets were uncoated, thus permitting diffuse reflectance.

Product A contained microcrystalline cellulose and lactose as the major excipients (95.4% total tablet mass). Additional excipients and dyes accounted for a further approximate 1.5% (w/w). Nominal bromazepam mass was 6 mg per tablet, equivalent to 3% (w/w). Nominal active content was therefore 30 mg g^{-1}. Samples were produced with active contents ranging from 20 to 130% of the nominal value. Concentrations were 20, 40, 60, 70, 80, 90, 110, 120 and 130% of the label value; 15 tablets were analysed at each concentration.

Product B contained microcrystalline cellulose, starch, lactose, and magnesium stearate. Nominal clonazepam weight was 2 mg per tablet, equivalent to 1.18% of total tablet mass. Thus nominal active content was approximately 11.8 mg g^{-1}. Concentrations from 70 to 130% of the label value (70, 80, 90, 110, 120 and 130%) were analysed ($n = 20$ per concentration). To produce homogeneous tablets no tablets were manufactured below 70% to prevent important differences from a tablet to another in the low content samples. In order to keep a high number of product B samples for the study the number of tablets was extended to 20 per concentration.

Tablets of both products containing the nominal amount of active were taken from regular production. Out-of-specification samples were prepared by the formulation development department using production department guidelines. The range of active content covered the decision range required by the US Pharmacopoeia for evaluating content uniformity tests. Excipients masses were corrected to obtain the same mass per tablet whatever the active concentration.

2.2. NIR measurement

NIR spectra were recorded on a NIRSystems 5000 Rapid Content-Analyser (Foss NIRSystems, Silver Spring, MD, USA) controlled with Foss software Vision (v 2.31). The reference (ceramic) was scanned 32 times. Each spectrum was the result of the mean of 32 scans measured with an increment of 2 nm between 1100 and 2498 nm. Figs. 1 and 2 show the raw spectra of products A and B, respectively. The samples were centred on the spectrometer window and the measurements run on several days. A reference was measured every 10 samples to document instrument stability. Both sides of every tablet were measured and the spectra pooled for processing as a single sample in all subsequent computations. Each tablet spectrum was assigned the corresponding concentration value obtained by the reference method. The recorded spectra were exported for computation as JCAMP files to a

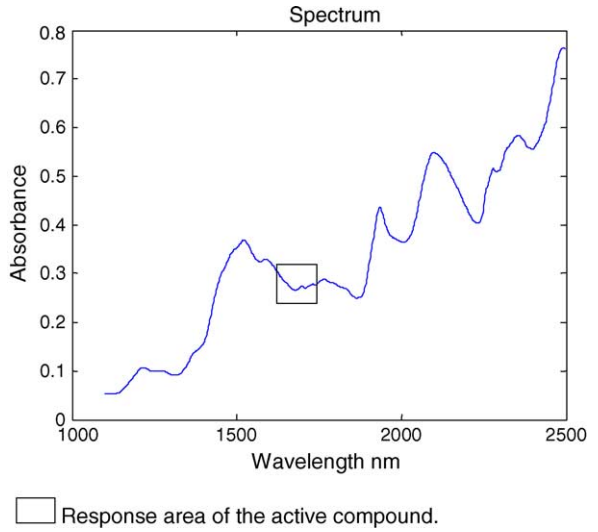

Fig. 2. Raw spectrum of product B.

Matlab environment (v 6.5 R13; MathWorks, Natick, MA, USA). All processing was performed in Matlab using PLS Toolbox v 3.0 (Eigenvector, Manson, WA, USA).

2.3. UV reference measurement

Both of the reference methods used for the different products are validated methods currently used for the quality control. As they are destructive methods they were performed on each tablet one by one after the NIR measurement.

Product A. The reference method was a UV cell measurement of absorbance at 285 nm, path length 10 mm, using an ATI UNICAM UV4 spectrometer (Spectronic Analytical Instruments, Garforth, UK). The solvent was prepared 24 h before use by mixing 51.0 g of sulphuric acid and the corresponding amount of LiChrosolv® methanol (Merck, Darmstadt, Germany) to obtain 10.0 L of solution. Each tablet was dissolved in 1.0 mL of deionised water and 29.0 mL of the prepared solvent. The solution was stirred for 10 min, then filtered on a membrane disc of 0.45 μm porosity. The 10.0 mL of this solution was diluted to 100.0 mL with the solvent.

2.4. HPLC reference procedure

Product B. A high performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA) was coupled to a UV detector fitted with a 10 mm cell for measuring absorbance at 242 nm. Each tablet was dissolved in 100.0 mL of 0.1N hydrochloric acid, sonicated for 10 min, and the solution stirred for 30 min before filtration through a 0.45 μm porosity membrane filter and transfer to an HPLC vial. The 20.0 μL samples were injected onto a nucleosil C18 column (5 μm, 4 mm i.d. × 25 cm) with mobile phase (30% (v/v) acetonitrile, 30% (v/v) methanol, 40% (v/v) water) running at a flow rate of 1.0 mL min^{-1} and pressure 230 bar.

2.5. Calibration and prediction

Statistics. Goodness of fit of a prediction model was evaluated according to the following criteria: low standard error of calibration (SEC), low standard error of prediction (SEP), high correlation coefficient (R^2), and low bias. SEC, SECV (standard error of cross-validation), SEP, bias, and SEP(c) (SEP with bias correction) were calculated by the Matlab software. The formulae and statistical strategy are described by Naes et al. [26].

2.6. Spectral data pretreatments

NIR spectra are affected by the state of the tablet surface [27]. The baseline can drift and maximum absorbance may change. Spectral pretreatments correct these interferences and instrument changes. Several standard pretreatments were used to minimise SEP. The methods are briefly described and references given:

- *Normalisations*:
  - Standard normal variate (SNV) [28] is a mathematical transformation of log(1/*R*) spectra. The spectral data are reduced and centred using the following formula:

$$\mathrm{SNV}_i = (x_i - \bar{x}) / \sqrt{\sum_i (x_i - \bar{x})^2 / (w - 1)}$$

    where i is the wavelength index, x_i the log(1/*R*) value at wavelength I, w the number of wavelengths in the segment, $\bar{x}$ the mean of log(1/*R*) on the segment, and SNV_i the corrected value of log(1/*R*) at wavelength i.
  - Multiplicative scatter correction (MSC) [29] improves the linearity of the relation between constituents and spectral values. This method was useful when applying linear regression methods as it eliminated scattering effects. A regression model was computed by the least square method $x_i = a + b\bar{x}_i + e_i$ (with a and b as the model coefficients and e_i the model error at wavelength i). Therefore the corrected values were calculated using the formula:

$$x_{i,\mathrm{corrected}} = (x_i - a)/b$$

- *Derivatives* enhance spectral information and reduce baseline drift. The Savitzky–Golay [30] second derivative was performed with a 5-point smooth on each side of computed point noted D2 in this study.
- *Orthogonal signal correction* (*OSC*) [31] removes spectral information irrelevant to calibration. It is first calculated on a calibration set (data matrix **X**) against the sample reference values (matrix **Y**) to remove concentration-extraneous information. This creates a correction matrix for all information orthogonal to the test matrix (matrix $\mathbf{T}^t\mathbf{P}$), matrix **T** being made orthogonal to matrix **Y**. In this case, the matrix will contain a correction coefficient to remove information orthogonal to the concentration of active. Corrected matrix $\mathbf{X}_{\mathrm{osc}}$ is therefore calculated as follows:

$$\mathbf{X}_{\mathrm{osc}} = \mathbf{X} - \mathbf{T}^t\mathbf{P}$$

  The correction matrix is then applied to all the new spectral data used for validation or prediction.

2.7. Regression methods

The influence of pretreatments was examined. Models were developed with various combinations of the mathematical pretreatments. They were validated by using the same data set. We also examined the influence of two standard regression methods, principal component regression (PCR) [26] and partial least squares (PLS) [32] regression, selected because they were the most accurate in terms of model complexity and the number of samples used to build the calibrations.

The optimal numbers of factors for PCR and PLS were determined by a cross-validation procedure with groups of two spectra (each side of the sample being represented by a spectrum).

2.8. Modelling

Total samples were divided into two sets. All calculations were performed on a calibration set and applied to a validation set, representing two thirds and one third, respectively, of the total samples, each sample comprising two spectra. A sample could not appear in both sets, meaning that spectral selection was designed block-wise. Wavelengths were not selected for the models; the whole range was used in the calculation. The models were built after cross-validating the sample sets, taking each sample, i.e. the spectra of both side of the tablet, out of the computation one after the other. The number of factors was selected by performing an *F*-test ($\alpha = 5\%$) on the standard errors of cross-validation (SECV). This *F*-test consists in searching the model with the lowest number of factors and that gives a SECV, which is non-significantly different from the lowest error calculated on the first twenty factors [33].

2.9. Method validation

The validation processes were achieved following the EMEA guideline [25]. The linearity was tested by evaluating the slope, the *y*-intercept and the correlation coefficient on the plot of prediction of active content by NIR versus values obtained with the reference method. As accuracy test, 12 and 20 production samples of products A and B, respectively, are measured by NIRS and with the reference methods. The average values for the different batches were compared by standard deviation. A *t*-test was computed on the NIRS prediction and reference values. The repeatability of each models are evaluated by measuring 10 times a production tablet by the same operator. The standard deviation and relative standard deviation are calculated on the predictions.

3. Results and discussion

3.1. Spectra

Bands of the actives in the NIR spectra of the two products are identified by comparison of the NIR spectrum of the pure actives, and of the placebos and of the production samples.

Fig. 1, the raw spectrum of product A, shows characteristic vibration bands from the active compound centred at 1380, 1650 and 2140 nm. Fig. 2 (product B) shows a specific active compound band centred at 1670 nm. Both spectra show water absorbance bands at 1450 and 1950 nm.

3.2. Model optimisation

The purpose of developing models is to be able to predict a sample property from an NIR spectrum as the sole raw data. For this reason the model must be as accurate but also as robust as possible, which usually involves compromise. The models are calculated on full range spectra for both products. No wavelength selection are performed. A model is built on a first data set called the calibration set. A second data set known as the validation set is used to test the model.

Table 1
Results of the PLS calculation on product A for the different pretreatments

	None	SNV	MSC	SNV + D2	MSC + D2	D2 + SNV	D2 + OSC
PC number	14	12	12	11	11	11	13
SEC (mg/tablet)	0.129	0.158	0.158	0.152	0.152	0.170	0.120
R_{cal}	0.9980	0.9970	0.9970	0.9972	0.9972	0.9965	0.9985
SEP (mg/tablet)	0.156	0.177	0.177	0.201	0.201	0.226	0.170
SEP(c) (mg/tablet)	0.156	0.177	0.177	0.200	0.200	0.206	0.169
Bias (mg/tablet)	0.005	−0.005	−0.005	0.013	0.013	0.013	−0.012
R_{val}	0.9968	0.9959	0.9959	0.9947	0.9947	0.9932	0.9962

R_{cal}: coefficient of correlation of the calibration; R_{val}: coefficient of correlation of the validation.

Table 2
Results of the PLS calculation on product B for the different pretreatments

	None	SNV	MSC	SNV + D2	MSC + D2	D2 + SNV	D2 + OSC
PC number	9	9	9	9	9	8	10
SEC (mg/tablet)	0.081	0.064	0.064	0.060	0.060	0.068	0.044
R_{cal}	0.9791	0.9867	0.9867	0.9885	0.9885	0.9852	0.9938
SEP (mg/tablet)	0.084	0.072	0.072	0.068	0.068	0.079	0.240
SEP(c) (mg/tablet)	0.084	0.072	0.072	0.068	0.068	0.079	0.083
Bias (mg/tablet)	−0.006	−0.001	−0.001	0.002	0.002	0.005	−0.224
R_{val}	0.9780	0.9836	0.9836	0.9856	0.9856	0.9806	0.9870

R_{cal}: coefficient of correlation of the calibration; R_{val}: coefficient of correlation of the validation.

Table 3
Results of the PCR calculation on product A for the different pretreatments

	None	SNV	MSC	SNV + D2	MSC + D2	D2 + OSC
PC number	12	11	11	10	10	5
SEC (mg/tablet)	0.192	0.216	0.216	0.250	0.250	0.225
R_{cal}	0.9954	0.9942	0.9942	0.9922	0.9922	0.9937
SEP (mg/tablet)	0.202	0.228	0.228	0.250	0.250	0.243
SEP(c) (mg/tablet)	0.200	0.226	0.226	0.248	0.248	0.242
Bias (mg/tablet)	0.027	0.030	0.030	0.025	0.025	−0.012
R_{val}	0.9947	0.9932	0.9932	0.9918	0.9918	0.9922

R_{cal}: coefficient of correlation of the calibration. R_{val}: coefficient of correlation of the validation.

Once the calibration and validation steps have been performed, the model is put to routine use by running NIR analyses in parallel with the officially approved method. The aim is to acquire long-term experience which can then be used to register the NIR method and the models on which it is based. Once approved, the models can be used routinely. This study describes the first and second steps only.

Selected spectral pretreatments were applied to both data sets using Matlab: SNV, MSC, SNV + D2 (SNV + 5-point second derivative Savitzky–Golay smooth either side of the computed point), MSC + D2, D2 + SNV, D2 + OSC. Tables 1 and 2 show the PLS results for products A and B, respectively. With product A, even if the statistics (SEP and bias) are of the same order of magnitude in all computations, the number of factors used by the model decreased depending on the pretreatment. Therefore to obtain similar reliability with the model fewer factors were needed with pretreatments than if none were performed. Hence pretreatments enhanced spectral information and reduced spectral variability, independently of the chemical properties. Tables 1 and 2 show that in both our cases the choice of SNV + D2 gave a better calibration than the D2 + SNV proposed by Fearn [34].

Table 3 shows the different results of PCR calculation with product A. By comparing Tables 1 and 3, we can conclude that both PLS and PCR could build a calibration from this data set. However, PLS gave lower SEPs than obtainable by PCR, and was therefore selected for this study.

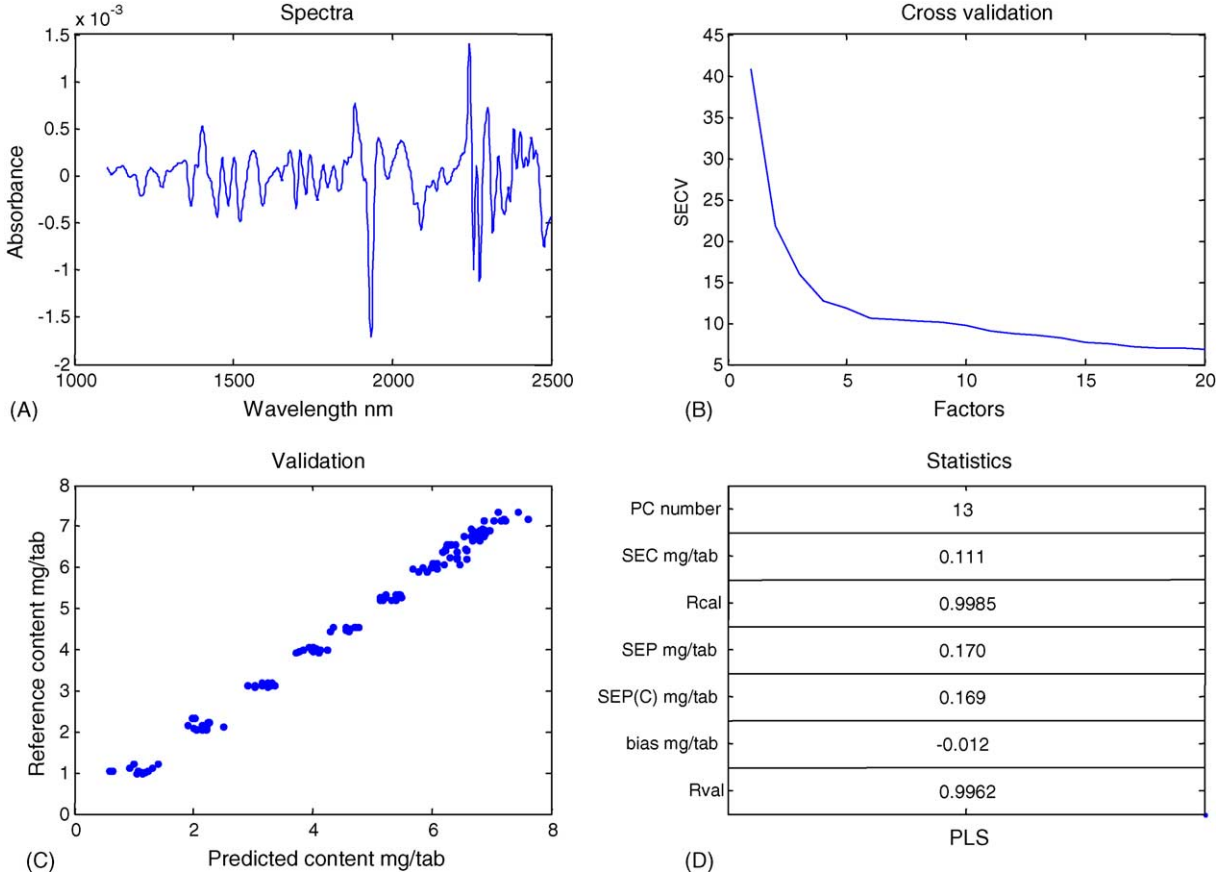

Fig. 3. Overview model with PLS regression on product A with a second derivative and an OSC as pretreatments.

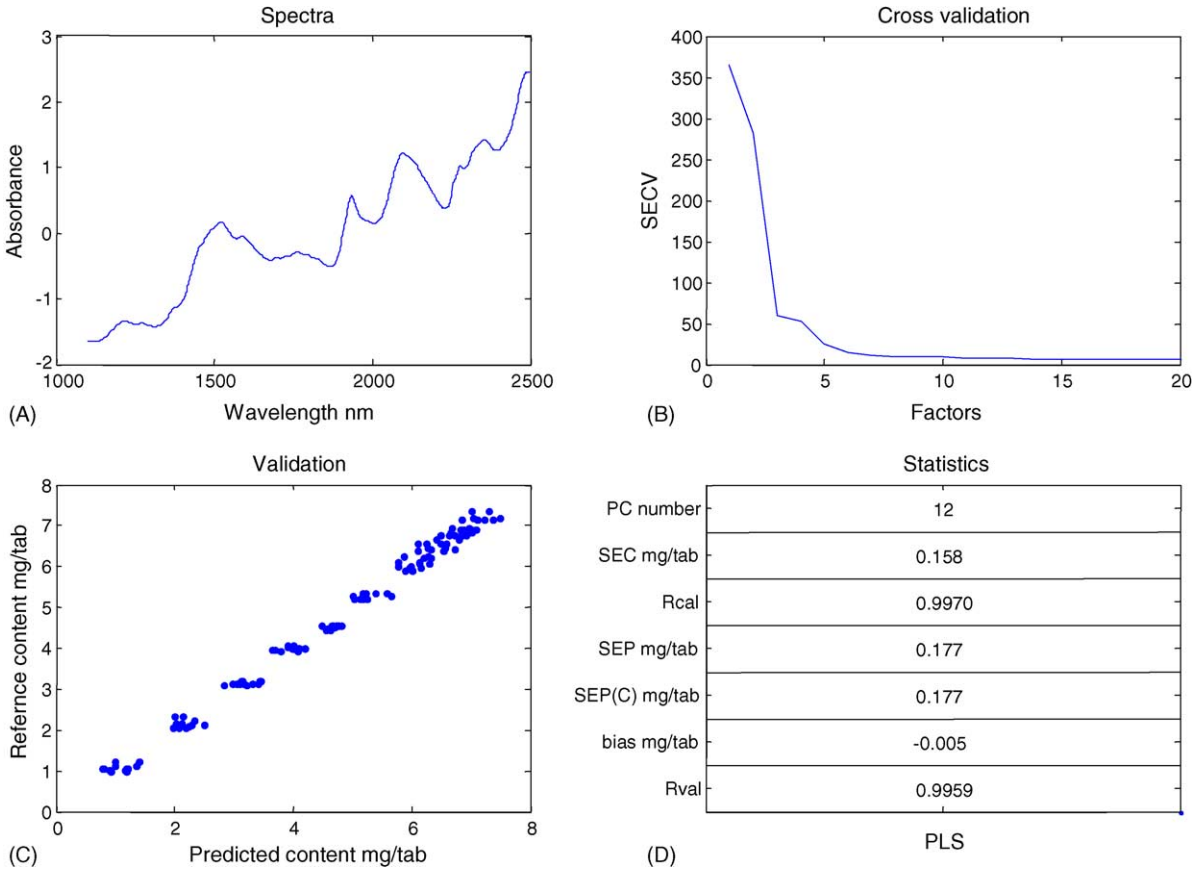

Fig. 4. Overview model with PLS regression on product A with SNV as pretreatment.

3.3. Product A

Product A calibrations were performed over a wide concentration range because the label content allowed the production of such tablets. The model with the lowest SEP was calculated from spectra pretreated with a second derivative followed by OSC. This model used 13 factors, achieving an SEP of 0.1699 mg and a bias of −0.0124 mg. The coefficient of correlation R^2 between UV measurement and NIR prediction was 0.9962 on the validation set. Fig. 3(C) shows the linearity and correlation between the two methods by plotting one against the other. The plot of the reference values against the NIR prediction of active content has a linear trend line with a slope of 0.998 and an intercept of 0.021. The loading plot of the coefficients (Fig. 3(B)) used by the model showed noisy coefficient responses, suggesting that the model was not robust enough for accurate prediction. For this reason a simpler pretreatment such as SNV was chosen. Even if the results were not showing such a strong correlation (Fig. 4(D)), we can see in Fig. 4(B) that the highest regression coefficients were calculated mainly at the active bands centred at 1380 and 1650 nm and that coefficients were less noisy to 2300 cm^{-1} and the relative standard deviation obtained for this model is 2.9%. Fig. 4(C) showed linearity between the NIR prediction of the active content and the reference values on the validation set, the slope of the linear trend line is 0.989 and its intercept is 0.043. The model using the SNV is preferred to the one using the MSC even if the statistics are the same, because this pretreatment does not depend of the sample set on which it is computed.

3.4. Product B

The most accurate model was built with nine PLS terms, and pretreatment with SNV and a second derivative (Fig. 5(D)). The regression coefficient of the model (Fig. 6) shows the importance of the active band centred at 1670 nm in the calibration. This model had an SEP of only 0.0682 mg with a bias of 0.0024 mg. It can also be seen (Fig. 5(C)) that the correlation between NIR prediction and HPLC measurement was 0.9856. The relative standard deviation is in this case of 3.4%. The pretreatment SNV + D2 is preferred to MSC + D2 because of his computing properties as the SNV was preferred to MSC for the precedent product. The influence of the specific band of the active at 1670 nm (Fig. 6) can lead to expect saving time employed in the analysis and improving the regression model by performing a wavelength selection.

The guideline published by pharmaceutical authorities for the validation of NIR methods [25] described the steps to

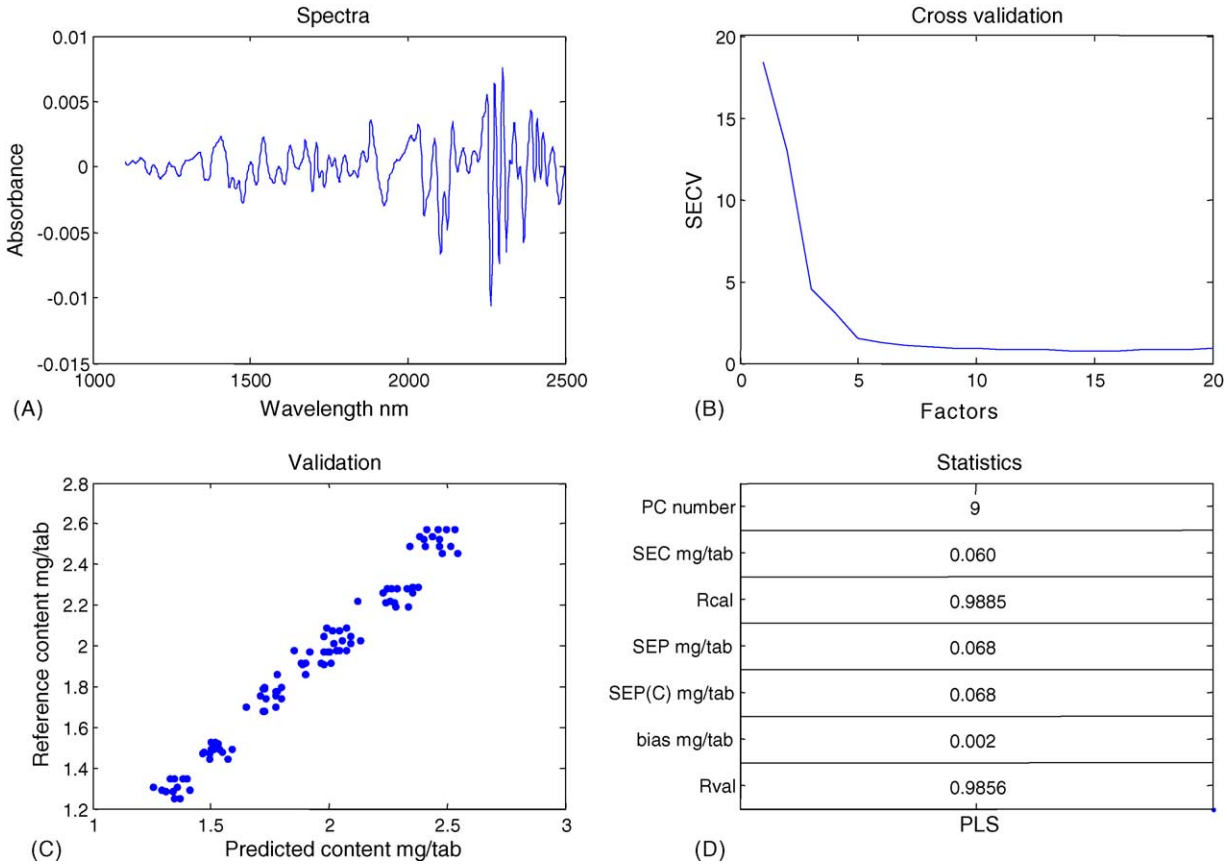

Fig. 5. Overview model with PLS regression on product B with a SNV and a second derivative as pretreatments.

follow to validate the use of NIRS by pharmaceutical industry. The purpose of this study was not to fully complete the validation as analytical method of the determination of active content in low-dosage tablet. Nevertheless parts of the validation protocol had been performed. The results of linearity, accuracy and repeatability are exposed in Table 4. These method validations showed that NIRS was accurate on production samples and repeatable. Results of calculation of standard deviation of the reference through SEP and RPD showed that NIRS is in both cases satisfying for quantification [35].

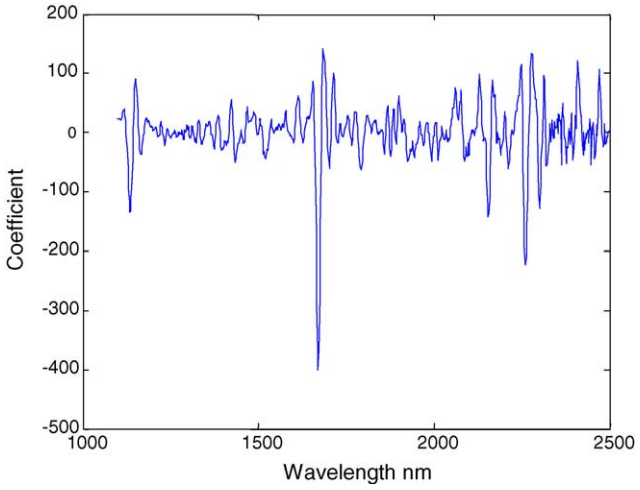

Fig. 6. Regression coefficient for product B with a SNV and a second derivative as pretreatments.

NIR is a rapid quantitative analytical method once it has been validated, but the requisite calibrations are time-consuming. It requires the production of a range of samples that differ from their commercial available counterparts solely in concentration. Model-building also requires an optimisation step, i.e. the choice of a regression method and spectral pretreatments strongly informed by the data set. Thus every new calibration must be optimised. A final point to bear in mind is that NIR calibrations include the errors of the reference methods mainly due to sample preparation. Once one has dealt with all these aspects, he can use NIRS as a far faster technique than HPLC for example, which as any method used in quality control also requires an optimization step. Moreover NIRS does not consume solvent as no sample preparation is necessary.

3.5. *NIRS and the pharmaceutical industry*

Diffuse reflectance NIR can provide information on active substance concentration at the sample surface. The result can then be converted to content, assuming a tablet size

Table 4
NIR prediction validation results for products A and B

Test	Description	Parameters	Product A	Product B
Linearity	NIR = a REF + b			
		A	0.989	0.935
		B	0.043	0.127
		Correlation	0.996	0.986
Accuracy	Paired t-test on NIR prediction and reference values on production batches		12 samples	20 samples
		Average difference[a] (mg)	0.015	0.007
		S.D. (mg)	0.118	0.055
		t_{exp}	0.615 (NS)	0.525 (NS)
		t_{crit}	2.201	2.09
	Production samples from three different batches	Nb. samples	10/10/10	10/10/10
		Average difference[a] (mg)	0.14/0.08/0.25	0.08/0.05/0.07
		S.D. (mg)	0.13/0.16/0.20	0.11/0.10/0.08
Repeatability	One production sample analysed 10 times by the same operator	NIR average (mg)	5.91	1.96
		S.D. (mg)	0.041	0.021
		R.S.D. (%)	0.70	1.19
	RPD[b]	$S.D._{ref}$/SEP	10.97	5.79

S.D.: standard deviation; R.S.D.: relative standard deviation; NS: non-significant.
[a] Average difference = bias.
[b] RPD: standard deviation of the reference through SEP (higher than 3: satisfying for quantification).

and mass complying with specification and a tablet of homogeneous mass. Tablets not complying with their mass specification must be removed before determining active content mass by NIR. This precondition is readily met in the quality control laboratory of a pharmaceutical manufacturer.

NIR predictions are not significantly different to reference method measurements. The main advantages of NIR are that it does not require sample preparation and it is a non-destructive method. Therefore it can be used in-line or at-line for a quality control of the production and for a better understanding of the process.

4. Conclusion

Quality control departments have already been using NIR for tablet identification. It would be a clear advance to combine this with an active content assay, so that qualitative and quantitative analysis can be performed by acquiring a single sample spectrum. Our study has shown the feasibility of generating calibration spectra for the quantification of active content in low-dosed intact tablets. The demonstration was performed using two different pharmaceuticals, and results were comparable to currently approved methods. Diffuse reflectance NIR has the potential to become a reliable and robust quality control method for determining active tablet content; it can even be proposed for total intact tablet quality control as admitted by the pharmaceutical authorities.

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