Talanta 147 (2016) 351–357

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Calibration transfer from powder mixtures to intact tablets: A new use in pharmaceutical analysis for a known tool

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ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
30 September 2015
Accepted 2 October 2015
Available online 9 October 2015

Keywords:
Calibration transfer
Double window piecewise direct standardization
Intact tablets
Near-infrared spectroscopy
Pharmaceutical quality control
Nevirapine

ABSTRACT

Calibration transfer is commonly used for spectra obtained in different spectrometers or other conditions. This paper proposed the use of calibration transfer between spectra recorded for the same samples in different physical forms. A new method was developed for the direct determination of nevirapine in solid pharmaceutical formulations based on diffuse reflectance near infrared spectroscopy (NIRS) and partial least squares (PLS). This method was developed with 50 powder mixtures and then, successfully extended to the quantification in intact tablets by using calibration transfer with double window piecewise direct standardization (DWPDS). This chemometric strategy provided good results with a small number of tablet transfer samples, only seven, prepared out of the narrow range of active principle ingredients (API) content around the nominal value of the formulation (100%). The method was fully validated in the working range of 83.0–113.9% of nevirapine and the use of DWPDS allowed to significantly decreasing the root mean square error of prediction (RMSEP) from 4.8% (tablets predicted by a model built with only powder samples) to 2.6%. The range of relative errors decreased from −5.1/8.7% to −4.6/3.3%. Considering that the amount of raw materials demanded for preparing tablets is up to ten times higher than for powder mixtures, this type of application is of particular interest in pharmaceutical analysis. In the context of process analytical technology (PAT), the use of the same multivariate model in different steps of the production is very advantageous, saving time and labor.

1. Introduction

The combined use of near infrared spectroscopy (NIRS) and chemometric tools of multivariate calibration has been consolidated as a viable alternative for the quality control of active ingredients in pharmaceutical formulations. NIRS provides simple, rapid, low cost and non-destructive methods, which require a minimum of sample pre-treatment and are environmentally friendly, without neither the consumption of solvents nor generation of chemical waste. In the last years, methods based on NIRS have been successfully applied for analysis in formulations of different physical forms, such as solutions, suspensions, powder mixtures and intact tablets [1–10]. Particularly stimulating for the development of NIRS methods was the Process Analytical Technology (PAT) initiative, issued by the Food and Drug Administration (FDA) [11]. It has opened perspectives for incorporating new technologies in the production and quality check of pharmaceutical products, considering the need of control of all the steps of the process, from the raw materials, going through the intermediates, to the final products.

Most formulations are available in solid forms, mainly tablets. For the production of tablets, a crucial intermediate step is the mixing of raw materials: active principle ingredients (API) and excipients. In order to analyze tablets, some analytical methods have simply added a step of pulverization and measured powder mixtures [5,10], with the drawbacks of increasing sample manipulation and analytical costs. Other methods have required the production of dozens of intact tablets with a range of chemical composition wider than the narrow API concentration interval around the nominal value. One example is a method developed based on the production of a mixed sample set including powdered samples plus tablets, in order to introduce production variability [1]. Other example is a slightly different approach, at which production tablets were ground and over or under-dosed

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http://dx.doi.org/10.1016/j.talanta.2015.10.006
0039-9140/

by adding known amounts of the formulation components [8]. However, as production samples have been used, a reference method to quantify the actual content of API must be employed. A more recent alternative, that has not demanded a reference method for the calibration samples, calculates the difference between the spectra of tablets and powder mixtures of identical composition prepared in the laboratory, generating a set of vectors that define the overall process variability [7]. In the present paper, it was proposed another alternative that also requires no reference method and uses fewer samples. The method is based on a new use for a known chemometric tool, calibration transfer.

A practical limitation to multivariate calibration occurs when an existing model is applied to spectra obtained under different instrumental, environmental or sample conditions. Even if identical samples were measured, the spectral variation of the two responses that is modeled by the method might differ [12]. If the sources of variation are known, every sample can be remeasured, demanding the reconstruction of a robust model. To avoid an expensive and time consuming full recalibration, the alternative of correcting instrumental and environmental differences by calibration transfer methods has been proposed [13]. The most common methods for correcting these differences are based on standardization of spectral responses, direct standardization (DS) and piecewise direct standardization (PDS) [12–14]. In these methods, the objective is to provide the same reading for a sample measured on a secondary (child or slave) condition as it does on the primary (parent or master) condition at which the calibration model was generated. DS directly relates spectral response matrix of samples measured in the primary condition, $\mathbf{S}_1$, to their responses obtained on the secondary one, $\mathbf{S}_2$, through a linear relationship described by the transformation matrix **F**, which is estimated according to Eq. (1),

$$\mathbf{F} = \mathbf{S}_2^{+}\mathbf{S}_1 \quad (1)$$

where $\mathbf{S}_2^{+}$ is the pseudo-inverse of $\mathbf{S}_2$.

F is typically estimated by means of PCR or PLS and subsequently used for projecting the secondary measurement space so that its property values can be predicted with the old model. A serious drawback of DS is that the number of samples is often much smaller than the number of channels involved in the regression, turning it prone to overfitting [12,14]. A way to circumvent this problem is to reduce the number of channels, which forms the basis of PDS. PDS is similar to DS, but incorporates the use of a moving window that steps across the variable range. For each wavelength of a sample spectrum, the absorbance values for the secondary condition are regressed against the corresponding values in a spectral window of neighboring wavelengths measured at the primary condition. PDS models may perform adequately where features are present in the transfer spectra, but not very well when featureless regions are frequent. Thus, a further modification has extended the PDS algorithm by incorporating a double window (DWPDS) [15]. DWPDS is based on a window data on both primary and secondary conditions used in the standardization, which increases the modeling flexibility. The form of the model is identical to PDS and some authors have considered it the best method for transferring NIRS models [16].

In the last paragraph, the emphasis in calibration transfer between samples measured at different conditions was purposefully adopted. However, most of published papers have been restricted to different instrument conditions [17–19] and a few ones have dealt with calibration transfer changing another condition, such as the temperature of measurements [20,21], or measurement time in milk analysis [22]. Considering that powdered samples present higher scattering than compressed ones [6], thus, affecting their NIR spectra, this paper proposed the calibration transfer between the same samples measured at different physical forms. In pharmaceutical analysis, this strategy is advantageous in terms of practicality, simplicity and costs reduction, since the same model can be used in different steps of the production and few samples of intact tablets outer of production specifications are required for constructing the model.

The analyte chosen for developing this methodology was nevirapine (NVP). NVP was the first nonnucleoside reverse transcriptase (RT) inhibitor used in clinical treatment of HIV disease and still being one of the most used medicines in HIV treatment, due to its robust virologic efficacy and a good safety profile [23]. In Brazil, NVP formulations are produced in governmental industries, due to its strategic importance for the public health. NVP has no quantitative reference method described in Brazilian Pharmacopeia [24], and a UV spectrophotometric method described for similar formulations is used as reference in local industry. In the literature, NVP has been determined in pharmaceutical formulations by chromatographic and electrophoretic methods, such as HPLC [25], HPTLC [26], MEKC [27] and CZE [28]. As far as it is known, NVP formulation has not been previously determined by a simple and direct NIR procedure. The NIRS method was initially developed and validated for powder mixtures and then, transferred to intact tablets by using the DWPDS method.

2. Materials and methods

2.1. Samples, design and spectra acquisition

All the chemical reagents used were of analytical grade, purchased from certified suppliers and used without further purification. The target formulation contains NVP, two major and five minor excipients. Two four-component experimental designs were built. The main design consisted of 78 samples that were prepared only as powder mixtures. The second one consisted of 9 transfer samples that were prepared as both powder mixtures and tablets. Random experimental designs were employed in order to avoid chance correlations and increase method robustness [2]. Random numbers within each component range were generated for each sample using Microsoft Excel®. The NVP content ranged from 80% to 120% of the nominal value of the target pharmaceutical formulation (200 mg of NVP per 800 mg, the average mass of one tablet) and two major excipients ranged independently from 95% to 105%. The other five minor excipients were modeled as one component design and their masses were kept constant in all samples. Due to the random values employed, the total weights of each sample were not constant and thus, the effective API contents were recalculated. The composition of all the samples of the main (1–78) and transfer (T1–T9) designs are shown in Table 1 of Supplementary materials. Due to industrial secrecy, the excipients composition is not shown.

For the main experimental design, 15 g of each powder mixture sample were produced. For the transfer design, 200 g of each sample were produced, from which 15 g were used as powder mixtures and the remaining powder was compressed as 13 mm

Table 1
Results of the optimization of PLS model through outlier detection.

Model	1st	2nd	3rd	Final[a]
Number of calibration samples	61	56	50	50
Number of LV	4	5	6	6
RMSEC (%)	4.4	1.9	1.1	1.1
RMSEP (%)	3.9	3.5	3.2	1.7

[a] The final model includes the calibration transfer and outlier detection for the validation set.

circular biconvex shape non-coated tablets. All samples were stored in amber flasks. Samples from seven different production batches were also supplied for method comparison.

All the spectra were recorded from 1000 to 2400 nm with 2 nm steps, and resolution of 8 cm^{-1}, as the average of 50 scans. The spectra of powder samples were obtained inside the amber flasks, after pressing the powder with the probe. Five spectra of each tablet sample were recorded, with a rotation between each spectrum, and an average was calculated. Five spectra of raw NVP were recorded and an average was used for spectral comparison.

2.2. Apparatus and software

The spectra were recorded on a PerkinElmer Spectrum 100N FT-NIR using a diffuse reflectance probe for solids. Data were handled using the MATLAB software, version 7.13 (The MathWorks, Natick, MA, USA). The Partial Least Squares (PLS) routine came from the PLS Toolbox, version 6.7.1 (Eigenvector Technologies, Manson, WA, USA), and a homemade routine was also employed for the detection of outliers [10].

2.3. Modeling

The 78 samples of the first design were placed in ascending API concentration order and systematically separated as 52 samples for the calibration set and 26 for the validation set. A calibration transfer requisite is that the primary condition must be part of calibration set. So, the transfer samples were separated as powders for the calibration set and tablets for the validation set. Thus, the calibration set was composed by 61 samples and the validation set by 35 samples. The nominal concentrations, as target percentage, were used as reference values.

PLS models were developed using random subsets cross-validation (7 splits). An evaluation of the most used pre-processing techniques for NIR spectra was carried out [29]. Data were mean centered. Outlier detection was performed with a routine, that evaluates leverage and residues of X and Y [30], for the calibration set, and Jackknife method for validation set [31].

2.4. Intact tablets prediction

The ability to predict API content of intact tablets was tested by three different approaches. Firstly, a direct analysis of the tablets by applying the former model (obtained only with powder samples) was performed. Secondly, a multiform model was developed with both powders and tablets by replacing the powder transfer samples in the calibration set by the corresponding tablet transfer samples (*e.g.*, powder sample T1 was replaced by tablet sample T1, and so on). Thirdly, calibration transfer with the DWPDS method was employed in order to model differences between tablet and powder spectra. Aiming at evaluating specifically the prediction of tablet samples, a validation subset was separated containing only the tablet samples. In this way, the error differences between these approaches were only related to tablet prediction.

2.5. Multivariate analytical validation

The developed method was validated by estimating figures of merit (FOM), such as trueness, precision, linearity, working range, selectivity, sensitivity, analytical sensitivity, bias and residual prediction deviation (RPD). Due to the high API content in this formulation, the estimation of limits of detection and quantification were not necessary. Trueness was evaluated by estimating relative errors: root mean square errors of calibration (RMSEC), and prediction (RMSEP), and the range of individual errors. Precision was evaluated at two levels, repeatability and intermediary precision, at three API content levels: low (91.6%), central (100.0%) and high (107.1%). Repeatability was evaluated through 6 replicates of each level in one day by the same analyst. Intermediary precision was estimated by the results of two distinct analysts in two different days. Linearity of the model was evaluated by the correlation coefficient (r) between reference and predicted values, obtained after the evidence of the random behavior of the residuals. This verification was carried out by applying the tests of Brown–Forsythe (homoscedasticity), Ryan–Joiner (normality) and Durbin–Watson (independency) [31]. Selectivity is the amount of spectral data used in the model, calculated as an average ratio between the norm of net analytical signal (NAS) and the spectrum norm of each sample of the calibration set. Sensitivity is calculated as the Frobenius norm of the NAS vector of the calibration samples. Analytical sensitivity (γ) is the ratio between sensitivity and instrumental noise. Instrumental noise was estimated as the pooled standard deviation of 16 replicates of the spectrum of the probe holder with non-reflective material tip. The inverse of the analytical sensitivity (γ^{-1}) is an estimate of the minimum content difference distinguishable by the method considering the random instrumental noise as the only source of error. Bias is an estimate of the systematic errors of the method, calculated only for the validation samples. RPD is the ratio of the natural variation of the samples and the size of probable errors occurring during the prediction. A better description of these FOM can be found elsewhere [9,10,32,33].

Another tool employed for validating the method was the accuracy profile based on the β-expectation tolerance intervals (β-TI). This tool has been originally proposed for univariate methods [34] and then, extended to multivariate calibration with a focus on NIRS methods for pharmaceutical quality control [35]. The β-TI can be used as a complementary visual decision tool to evaluate the predictive performance of the method. They guarantee that a ratio β (*i.e.* 95%) of all the future results will present an error within the acceptance limits; thus, assuring that the proposed method fulfills all the requirements needed for validation. The interval is estimated using the Eq. (2),

$$\beta - \mathrm{TI} = RE(\%)_j \pm t\sqrt{1 + \frac{1}{pnB_j^2}}\,RSD(\%)_j \tag{2}$$

where p is the number of series of measurements, n is the number of independent replicates per series, RE(%) is the mean relative error from n replicates in the j_{th} level, RSD(%) is the relative standard deviation from n replicates in the j_{th} level. t is the t-student critical value for ν degrees of freedom. ν is calculated according to Eq. (3),

$$\nu = \frac{(R_j+1)^2}{\frac{(R_j+\frac{1}{n})^2}{p-1} + \frac{1-\frac{1}{n}}{pn}} \tag{3}$$

where R_j is the ratio between within series variance and between series variance, and B_j is estimated using R_j.

$$B_j = \sqrt{\frac{R_j+1}{nR_j+1}} \tag{4}$$

2.6. UV reference method

In order to complete the validation of the proposed methodology, real samples from seven production batches were analyzed by the developed NIRS method and compared with the results from the UV method officially used in the quality control of the industry (FUNED). A Shimadzu UV-1800 spectrophotometer was used. The measurements were performed at 283 nm in a 10 mm

quartz cell and the sample solutions were prepared in HCl 0.1 mol L^{-1}. A pool of 20 tablets of each batch was obtained and an average tablet weight was calculated [24]. Tablets are powdered and a fraction of the mass was solubilized in HCl 0.1 mol L^{-1}, filtered and diluted in HCl. Then, the absorbance was measured and compared with a standard solution absorbance.

3. Results and discussion

3.1. Model construction

NIR spectra of all the 96 samples, powder mixtures and intact tablets, are displayed in Fig. 1. In these spectra, non-linear baseline deviations are observed, due to the multiplicative light scattering. Thus, multiplicative scatter correction (MSC) was used as preprocessing. The best predictive model was then obtained by combining MSC with Savitsky–Golay (15 points in filter and second order polynomial fit) 1st derivative as evaluated by the lowest root mean square error of cross-validation (RMSECV). Variable selection with iPLS was performed, but there was no improvement in the results. So, the models were built using the full spectra, from 1000 to 2400 nm. Then, optimization of the model by outlier detection was performed in the calibration set.

To avoid the snowball effect [30], the outlier detection was performed only two times (three models). As the validation set will be used for making a comparison between intact tablets approaches, no outlier detection was performed in this step. Table 1 presents the number of calibration samples, latent variables (LV), RMSEC and RMSEP for each step of the optimization and a final model that includes results of calibration transfer and outlier detection in the validation described in Section 3.3. Eleven samples were detected as outliers in the calibration set. Two transfer samples were excluded as outliers, and this will affect the transfer model subsequently. The final PLS model was built with 6 LV and accounted for 96.4% of the total variance in X and 97.3% in Y.

As it was expected, a high correlation was observed between the API content and the first LV scores. Thus, a comparison between the LV1 loadings and the raw NVP preprocessed spectrum was performed (Fig. 2) in order to obtain a qualitative picture of the method's selectivity, as recommended by the European Pharmacopoeia [36]. In Fig. 2, major correlations were observed in the regions between 1100–1180, 1600–1740 and 2100–2260 nm. In these regions, the original bands centered at 1140, 1680 and 2230 nm correspond to the second and first overtones of C–H stretching, and the combination band of =CH and C=O stretching of the NVP molecule, respectively. A combination band of N–H+C–H was also presented at 2160 nm and an aromatic C–H first overtone at 1630 nm [37].

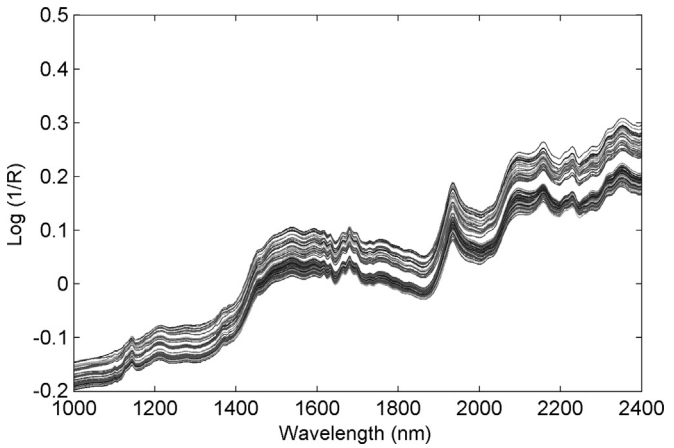

Fig. 1. NIR diffuse reflectance raw spectra (1000–2400 nm) of 96 nevirapine formulation samples with API content ranging from 83.0% to 113.9%.

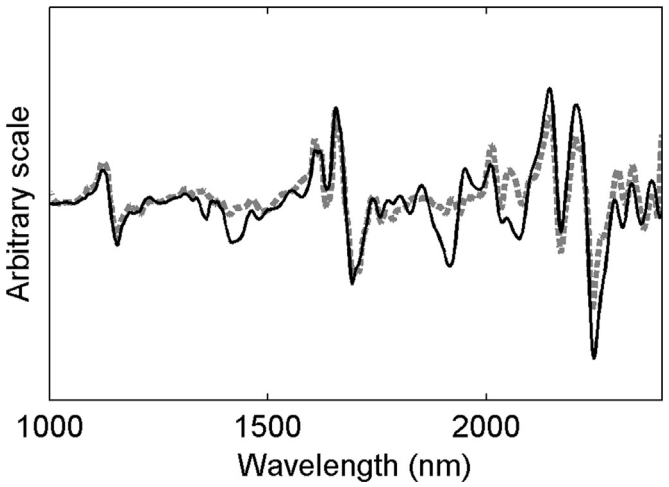

Fig. 2. Comparison between the loadings of LV1 (solid line) and the first derivative of the raw nevirapine spectrum (dashed line).

3.2. Intact tablets prediction and calibration transfer

Usually, the transfer samples are selected within the calibration set using an algorithm for a homogeneous and representative screening, such as Kennard–Stone [38]. However, as the transfer samples must be the same in both conditions, it is mandatory that the same powder-mixtures used to produce intact tablets be included in the calibration set. For this, the transfer samples must be selected before the model is built. Then, an experimental design that covers the full range of API content was the alternative for selecting the transfer sample set. The number of transfer samples is also an important choice. Bouvesse and Massart [38] have evaluated the effectiveness of calibration transfer models as a function of the number of the transfer samples, concluding that 3–4 samples are enough when using Kennard–Stone as the sample selection method and 6 samples when this selection is based on the sample leverages. Some papers have also prescribed the selection of about 10% of the calibration samples [39,40]. In this paper, 9 powder-mixture transfer samples were designed to build the transfer model, chosen as representative of the whole analytical range. This number was reduced to 7, because two of them were excluded as outliers.

The spectra of the transfer samples were preprocessed before the calibration transfer, so the powder model must be rebuilt using previously preprocessed data. For predicting the intact tablets, three approaches were compared. Firstly, tablets were predicted by a model built only with powder samples (powder model). Secondly, a model with powder plus tablet samples was built. This is a biased model, because the predicted tablet samples were also used in the calibration. Finally, the calibration transfer between powder and tablets were tested in order to obtain a robust model. The RMSEP for the prediction of intact tablets (RMSEPt) and the range of individual errors (Table 2) were used to compare the three different approaches.

Table 2
Comparison of three different approaches for the prediction of intact tablets.

Model	RMSEPt (%)	Error range (%)	
Powder	4.8	−5.1	8.7
Powder+tablet	3.3	−2.8	6.3
Powder (DWPDS)	2.6	−4.6	3.3

The direct prediction of tablet samples by the powder model presented the highest errors and the highest Q residues (spectral residuals), indicating that these samples were not well modeled. The model built with both powders and tablets presented lower errors than the direct prediction by the powder model. However, the overall calibration of this model was affected, since its RMSEC increased from 1.1% to 2.2%. In spite of being a biased model, with the predicted tablet samples showing lower Q residues than the average of the calibration set, it provided higher errors than the calibration transfer model.

For the optimization of the DWPDS model, windows ranging from 7 to 15 variables in the primary condition and from 9 to 21 variables in the secondary condition were tested. The best model was obtained with window sizes of 15 and 11 variables for primary and secondary conditions, respectively. This model provided a RMSEPt of 2.6% and individual errors ranging from −4.6% to 3.3%. These prediction errors are about half of the ones provided by the powder model. The Q residues of tablet samples were also decreased to almost the same level of the calibration samples. An *F* test at 90% confidence level ($F_{7,7}=2.78$) allows to confirm that there was a significant decrease in the RMSEPt of the calibration transfer as compared to the powder model ($F_{calc}=3.26$). However, there was no significant difference in comparison with the biased powder+tablet model ($F_{calc}=1.56$).

3.3. Multivariate analytical validation

As the calibration transfer improved the results, outlier detection was performed in the full validation set (including transferred tablets) with the Jackknife test. Then, 5 samples were excluded, only one of the transferred tablets. The final model was composed of 50 samples in the calibration set and 30 samples in the validation set. Calculated FOM are shown in Table 3. The analytical validation was carried out in order to obtain harmonization with the guidelines of ANVISA (Brazilian National Health Surveillance Agency) [41]. These guidelines, in turn, are based on the ICH ones [42,43].

Although there are no acceptable limits for trueness in these guidelines, the range of individual prediction errors (including powder and tablet samples) was considered small and acceptable, with all values within ±3.9%. In addition, both the mean

Table 3
Parameters calculated for evaluating FOM of the developed method.

FOM	Parameter	Value
Trueness	RMSEC (%)	1.1
	RMSEP (%)	1.7
	Relative errors (%)	−2.7/3.9
Precision	RSD repeatability (%)	1.5/1.2/3.0[a]
	RSD intermediate precision (%)	1.5/1.7/4.2[a]
Linearity	Durbin–Watson	1.84
	Slope	0.97 ± 0.02
	Intercept	2 ± 2
	Correlation coefficient	0.99
Working range	(%)	83.0–113.9
Selectivity	(%)	6.7
Sensitivity		0.0191[b]
Analytical sensitivity (γ)	($\%^{-1}$)	19.28
γ^{-1}	(%)	0.05
Bias	(%)	−0.8 ± 2.6
RPD	Calibration	4.2
	Validation	4.5

[a] Values for low (91.5%), mid (100.0%) and high (107.1%) levels.
[b] Values expressed as the ratio between log (1/*R*) and percent.

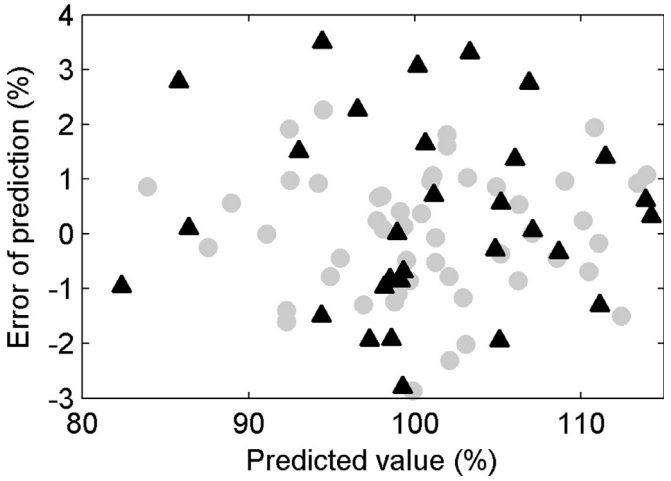

Fig. 3. PLS residuals plot for the calibration (circles) and the validation (triangles) samples.

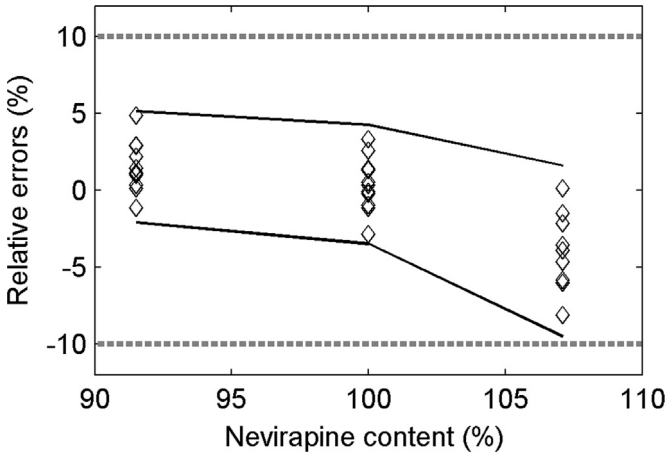

Fig. 4. β-expectation tolerance interval for the developed model. Estimated limits at 95% confidence level (solid lines), and maximum acceptable relative errors (dashed lines).

parameters RMSEC and RMSEP were below 2.0%. For evaluating precision, a maximum RSD of 5% is acceptable. Since all the RSD values were below 4.2%, the model was considered precise at both the levels, repeatability and intermediary precision. The linearity can be evaluated by a correlation coefficient of 0.99 for the calibration set, which was in accordance with the ANVISA guidelines. The randomness of the residuals, which can be visually checked in Fig. 3, was statistically confirmed at 95% confidence level, assuring the linearity of the model. For this, their normality, homoscedasticity, and independency were confirmed by applying sequentially the Ryan–Joiner, Brown–Forsythe and Durbin–Watson tests [31,32]. The estimate of 1.84 for the Durbin–Watson test confirmed the absence of autocorrelation in the residuals, since the acceptable range is between 1.5 and 2.5. Considering the linearity and accuracy (trueness plus precision) of the method, its working range was established between 83.0% and 113.9%, covering the acceptable range from 90% to 110% for NVP content in this type of pharmaceutical formulation.

In contrast to univariate calibration, requiring a minimum acceptable limit for selectivity is not of practical utility for multivariate methods, since even low selective models can be accurate. In our case, 6.7% of analytic signal were used for predicting NVP. However, this FOM is estimated as an average; thus, selectivity is only useful within a certain group of samples of similar qualitative composition. Considering that the sensitivity itself is not

Table 4
Determination of real samples (intact tablets) from seven different batches by the UV reference method and by the three tested approaches for the proposed NIR method. Units are in % of the target NVP content of the formulation.

Batch	1	2	3	4	5	6	7
UV	103.69	101.08	102.06	101.13	103.76	100.19	100.27
Powder −NIR	85.5	93.9	90.2	87.7	91.0	94.4	93.5
Powder+Tablets-NIR	92.7	92.3	93.0	87.5	84.6	91.9	89.1
DWPDS-NIR	97.2	101.6	98.0	94.3	101.8	101.1	102.8

appropriate for making a comparison between methods, γ was also calculated from the estimate of the instrumental noise, $\log(1/R)=0.00099$. The inverse of γ, 0.05%, was used to define the number of decimal places used to express the prediction results of the method. Although this estimate indicated the utilization of two decimal places, the more realistic use of only one decimal place was adopted for expressing the results.

The estimate of bias, $-0.8 \pm 2.6\%$, was not significantly different from zero by a *t*-test with 30 degrees of freedom and at 95% confidence level. Thus, the developed method can be considered free of systematic errors. Since RPD values higher than 2.4 are an indication of good NIR models [44], their estimates in Table 3 express the adequate predictive power of the developed model for both the calibration and validation sets.

β-TI was estimated at three levels, low, medium and high, all within the acceptable content range for NVP (90–110%), with two series of sextuplicates ($p=2$ and $n=6$) at 95% confidence level. Two replicates were detected as outliers by a jackknife test and eliminated. In spite of the observation of a negative bias at the higher level, the accuracy profile displayed in Fig. 4 shows that the confidence interval for the predictions is within the acceptable limits of $\pm 10\%$ of relative error. Thus, it is expected that 95% of predictions provided by the method will be within these practical limits adopted by the industry.

3.4. Analysis of real samples

In order to evaluate the robustness of the calibration transfer model, an external validation set was analyzed. This validation set consisted of real samples of intact tablets obtained from seven different batches, representing a period of one year of the production. Results were compared with the reference UV method. A paired *t*-test with seven degrees of freedom demonstrated that there is no significant difference between the two methods at 95% confidence level ($t_{calc}=1.58 < t_{crit}=2.365$). As shown in Table 4, the predictions for these same samples with the other two tested approaches for the NIR data clearly resulted in significant differences in relation to the reference method (powder $t_{calc}=6.39$, powder+tablet $t_{calc}=7.91$). For these real samples, RMSEP for the prediction of real samples (RMSEPr) was estimated as 4.1% for the DWPDS model, being much better than 11.6 and 12.1%, RMSEPr for the other two approaches, powder and powder+tablet model, respectively. Thus, these results proved the effectiveness of the developed calibration transfer model.

4. Conclusion

This paper developed and validated a simple, rapid and non-destructive multivariate NIR method for determining nevirapine in pharmaceutical formulations. This method was initially developed with powder mixtures and then, successfully extended to the quantification in intact tablets by using calibration transfer with DWPDS. This chemometric strategy provided good results with a small number of tablets prepared out of the narrow range of API content around the nominal value of the formulation. To the best of our knowledge, this is the first time that calibration transfer is used for spectra of the same samples in different physical forms. Considering that the amount of raw materials demanded for preparing tablets is more than ten times higher in comparison to powder mixtures (about 200 g *versus* 15 g), the elimination of a full recalibration for extending the model from powders to tablets is very advantageous, saving time and labor. In addition, the use of the same multivariate model in different steps of the production is particularly important in the context of PAT [11], simplifying and streamlining the quality control in pharmaceutical production, with the possibility of real time analysis. The extension of this strategy can also be suggested to tablets that have a more complex production, involving the control of other intermediate steps, such as granulation and covering. Finally, beyond the presented application in pharmaceutical analysis, the use of calibration transfer between spectra of samples in different physical forms can be suggested for other matrices, such as *in-natura* and pulp fruit or intact and sawdust wood.

Acknowledgement

The authors are grateful to CAPES and FAPEMIG for funding this research; and also to *Laboratório de Ensaios de Combustíveis* (LEC-UFMG, Belo Horizonte) for allowing the use of a NIR spectrometer.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2015.10.006

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