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DIRECT AND SIMULTANEOUS QUANTIFICATION OF ATORVASTATIN AND AMLODIPINE IN TABLETS BY NIR SPECTROSCOPY

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ABSTRACT. Near infrared spectroscopy (NIRS) is a technique widely used for direct and non-destructive analysis of solid samples. A NIRS method for the simultaneous quantification of atorvastatin and amlodipine in fixed-dose combination tablets was developed and fully validated. The PLS calibration model was developed based on the 26 samples prepared according to a D-optimal experimental design with 2 factors and 5 levels. The best predictive model for atorvastatin was developed using standard normal variate pre-processing method, 7 PLS factors; the best predictive model for amlodipine was developed using first derivate followed by standard normal variate pre-processing method and 7 PLS factors. The method was validated in terms of linearity, trueness, precision and accuracy. The validation results show that the method is reproducible, precise and has good accuracy and linearity profiles. Furthermore, comparative data obtained on independent samples shows no statistical difference ($p>0.05$) between the results predicted by the NIRS method and the values obtained using HPLC reference method. So, NIRS based on PLS multivariate calibration could be a suitable tool for the non-destructive, direct and simultaneous prediction of the chemical composition of a fixed-dose combination that includes two APIs in a single tablet and is helpful in achieving the goals of Process Analytical Technology (PAT).

Keywords: *near infrared spectroscopy, simultaneous quantification, amlodipine, atorvastatin, chemometry*

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INTRODUCTION

A single-pill combination drug is a fixed-dose combination (FDC) that includes two or more active pharmaceutical ingredients (APIs) combined in a single dosage form. FDC can benefit patients through the potential increase in efficacy and/or a reduced incidence of adverse effects, the convenience in terms of administration and compliance and potentially lower costs of manufacturing compared to the costs of producing separate products administered concurrently [1]. The amlodipine/atorvastatin single pill has been shown to improve patients‘ achievement of national-guideline-recommended blood pressure and lipid target levels and exhibits a safety profile consistent with its parent compounds. This combination pill is now available in Europe in formulations containing either 5 or 10 mg amlodipine and 10 mg atorvastatin [2,3].The combination is indicated for patients suffering from both high blood pressure and high levels of cholesterol and has had worldwide sales of more than $600 million in 2013.

The manufacturing process typically involves several unit operations, such as blending, granulation, tableting, and coating, all of which can have critical influences on the final quality of the product. Process monitoring is a methodology that guarantees a high-predefined quality standard and offers the possibility to react during the process if any parameters drift from the normal operating range, but it requires quick methods. Process monitoring is the goal of Process Analytical Technology (PAT). PAT is defined by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) as a concept which implies the design and control of pharmaceutical processes through real time measurements of critical process parameters that could affect quality assurance [4,5]. Direct analysis of intact solid dosage forms as whole tablets is considered to be a very important goal for NIRS analysis in the pharmaceutical industry, with increasing needs of on-line or in-line testing for process monitoring according to PAT [6,7].

In the field of APIs quantification in tablets, including fixed-dose combinations, the high performance liquid chromatography (HPLC) technique is widely used due to good selectivity, specificity and linear range [8]. Until now, only HPLC methods were developed and validated for the simultaneous quantification of both APIs (atorvastatin and amlodipine) in fixed-dose combinations [9,10]. However, this requires sample preparation, chromatographic separation of the analytes and takes hours, so therefore it is currently only done off-line [6,7,11]. Near InfraRed Spectroscopy (NIRS) is a non-destructive technique that allows the direct quantification of chemical properties such as the active pharmaceutical ingredients content [6,7,12] or physical characteristics as pharmaceutical properties [13,14] of tablets and powder blends for tableting

[11,15,16]. NIRS can be used to perform quantitative analysis of one, two or more compounds in different matrices, like pharmaceutical powder blends for tableting or tablets. The advantages of the NIRS method are numerous: non-invasive and non-destructive techniques, no sample preparation, high frequency of spectrum acquisition, as well as a large number of molecules which could be quantified [6]. Many papers are reporting the determination of API content in tablets by NIRS methods [12,17], but only a few are focusing on the prediction of two or more APIs and/or excipients in the composition of powder blends or tablets [6,7,18].

The aim of this work was to develop and validate a NIRS method for direct and simultaneous quantification of amlodipine and atorvastatin in tablets, using a direct, fast, non-invasive and non-destructive technique that requires no sample preparation.

RESULTS AND DISCUSSION

The NIR spectra of tablets contain both chemical information related with APIs and excipients contents and physical information related with tablet compaction. Therefore, pre-processing methods and wavelength selection ranges should be carefully chosen to extract the chemical information that is mainly correlated with the APIs concentration, in order to develop robust calibration models.

Spectra investigation

The calibration model was built after recording twenty spectra of each tablet formulation. Overall 520 tablets spectra were recorded and analyzed for the calibration model development (Figure 1). The NIR spectra of pure APIs is also presented (a1 – atorvastatincalcium; b1 – amlodipinebesilate) in the same figure.

Development of calibration models

The development of calibration models for APIs assay is an iterative technique and consisted in checking the predictive ability of several spectral pre-processing methods in association with different spectral regions with high NIR absorbance of the APIs of interest. By applying different spectra pre-processing methods in combination with different spectral regions a large number of models was generated. Among these, the most potentially interesting 6 models for each API were selected and presented in Table 1.

Table 1. Statistical parameters and number of principal components for atorvastatincalcium and amlodipinebesilate, without data pre-processing as well as after different spectra pre-processing

Atorvastatincalcium						
Pre-processing method*	none a	SLS b	SNV C	FD d	SLS-4 e	SNV-4 f
Spectral range (cm^{-1})**	R1	R1	R1	R1	R4	**R4**
Number of PLS factors	8	7	7	7	7	**7**
R^2	0.9408	0.9411	0.9423	0.9349	0.9586	**0.9588**
RMSECV (% w/w)	0.353	0.352	0.348	0.374	0.295	**0.292**
Bias	-0.00015	-0.00026	0.000301	-0.00010	-0.000477	**-0.00025**
Amlodipinebesilate						
Pre-processing method*	none g	MSC h	SLS i	SNV j	FD+SLS k	FD+SNV l
Spectral range (cm^{-1})**	R1	R1	R1	R1	R1	**R1**
Number of PLS factors	9	8	8	8	7	**7**
R^2	0.9675	0.9737	0.9733	0.9738	0.9757	**0.9768**
RMSECV (% w/w)	0.353	0.318	0.320	0.318	0.306	**0.299**
Bias	-0.000073	-0.00074	-0.00054	-0.00034	-0.000592	**-0.000551**

*none-no pre-processing, SLS-straight light substraction, SNV-standard normal variate, FD-first derivate, MSC-multiplicative scattering correction, FD+MSC – first derivative followed by multiplicative scattering correction, FD+SNV – first derivative followed by standard normal variate

**R1-Spectra range 1 region: 10000-4200cm^{-1}; R4-Spectra range 4 region: 10000-8270; 7700-7120; 6800-5616; 5400-4243 cm^{-1}.

In the case of the atorvastatincalciumcalibration, based on the analysis of different calibration models, the models generated using 4 spectra range had the best results. As the R^2 values of the models are very close, this parameter does not allow any clear differentiation between the models. Regarding RMSECV, the (f) model seems to be slightly better than the (b), (c) and (e) models: its RMSECV is the smallest. Thus, (f) was the selected model which was the most fitted for this purpose, and the model candidate for method validation according to current pharmaceutical requirements. The shape of the spectra after pre-processing according to this model is presented in Figure 1, a_2. In the case of the amlodipine besilatecalibration, the results look very similar, R^2 values of the models are very close, so the models were selected based on RMSECV values. Regarding RMSECV, the (l) model seems to be slightly better than the (h), (i), (j) or (k) models: its RMSECV is the smallest. The shape of the spectra after pre-processing according to this model is presented in Figure 1, b_2. The predictive ability of the chosen models was checked on independent samples in the validation step.

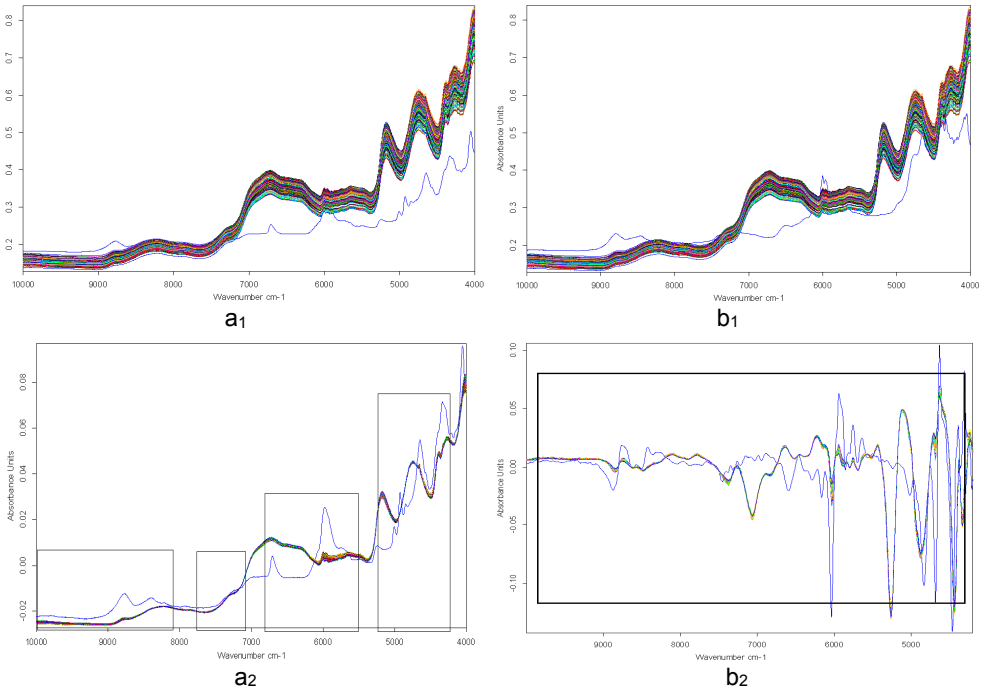

Figure 1. NIR spectra of tablets without preprocessing (a_1, b_1) and pre-processed using SNV-4 method (a_2) for atorvastatin calcium and FD+SNV method for amlodipine besilate (b_2); blue line in a_1, a_2– atorvastatincalcium; blue line in b_1,b_2 - amlodipine besilatespectrum

Validation of the method

Validation was based on ICH Q2 guidance and included linearity, range of application, accuracy, precision (repeatability and intermediate precision), and was done on validation samples. Independent validation samples similar to the calibration samples were prepared at 3 different active content levels (corresponding to 80, 100 and 120% concentrations) of each API (formulation N7, N13, N19, Table 4). The predictive performance of the chosen models was evaluated based on accurate profiles computed on the external validation samples. Accuracy represents the total error concept which is the sum of the trueness (systematic error) and precision (random error) and was evaluated by determining the accuracy profile following the methodology proposed by Hubert *et all* [23, 24]. Table 2 shows the validation results obtained with the developed NIR model (f, SNV-4) for atorvastatincalcium (standard normal variate pre-processing, 7 PLS factors and 4 spectral regions: 10000-8270; 7700-

7120; 6800-5616; 5400-4243cm^{-1}) and model (I, FD+SNV) for amlodipine besilate (first derivative followed by standard normal variate pre-processing, 7 PLS factors and 1 spectral regions: 10000-4200cm^{-1}).

Table 2. Validation results of the NIRS method

for the quantification of **atorvastatin calcium (f, SNV – 4)**						
Conc. level (mg/tablet)	Trueness		Precision		Accuracy	
	Relative bias (%)	Recovery (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	Relative tolerance limits (%)	Tolerance limits (mg/tablet)
9.31	-0.568	99.43	1.54	1.65	[-4.50, 3.37]	[8.89, 9.62]
10.34	1.475	101.48	0.99	1.09	[-1.13, 4.08]	[10.22, 10.77]
11.37	-0.091	99.91	0.50	0.80	[-2.57, 2.38]	[11.08, 11.64]
for the quantification of **amlodipine besilate (I, FD+SNV)**						
Conc. level (mg/tablet)	Trueness		Precision		Accuracy	
	Relative bias (%)	Recovery (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	Relative tolerance limits (%)	Tolerance limits (mg/tablet)
12.48	-0.823	99.18	1.33	1.49	[-4.48, 2.84]	[11.92, 12.83]
13.87	0.498	100.50	1.03	1.17	[-1.84, 2.83]	[13.61, 14.26]
15.26	-0.257	99.74	1.83	2.11	[-4.40, 3.88]	[14.59, 15.85]

The trueness of the method was evaluated by calculating the relative bias and the recovery. The recovery had very good values (close to 100%) for both APIs at all three concentration levels. The minimum value was 99.18 at a low concentration level of amlodipine besilate and maximum 101.48 at a medium concentration level of atorvastatincalcium. The precision evaluated as repeatability (intra-day precision) and intermediate precision (repeatability over different days) shows also good values for both APIs and at all concentration levels. The best repeatability and intermediate precision values were obtained at medium concentration levels for both APIs.

Figure 2 shows the linearity profile and the accuracy profiles of the NIRS method. The linearity profile of the method was evaluated by plotting the determinate concentration of APIs in validation samples by NIRS method as a function of introduced concentration. The linearity profile of the NIRS method for both APIs is shown in Figure 2 (left).The dashed limits on the graph correspond to the accuracy profile and the dotted curves represent the acceptance limits at ±5% expressed in the concentration unit per tablet. As seen in the Figure 2, the R^2 and the slope values are close to 1 for both APIs, confirming the linearity of the method for the direct and simultaneous quantification of atorvastatin and amlodipine in tablets.

For the accuracy profile the acceptance limits were set at ±5%, as required for the determination of API in pharmaceutical products [13].The β-expectation tolerance limits should be included in the acceptance limits. As seen in Figure 2, the β-expectation tolerance limits are fully included within the ±5% acceptance limits for both APIs, so it can be concluded that the NIRS method provides results with adequate accuracy for simultaneous atorvastatin and amlodipine assay, in tablets without any sample preparation over the range of 9.31-11.37 mg/tablet for atorvastatincalcium and 12.48-15.26 for amlodipinebesilate. The largest relative tolerance limits for atorvastatin calcium (-4.50%, 3.37%) were at the lowest concentration level and the largest relative tolerance limits for amlodipine besilate (-4.40%, 3.88%) were at the highest concentration level. The best accuracy was obtained at the medium concentration level for both APIs.

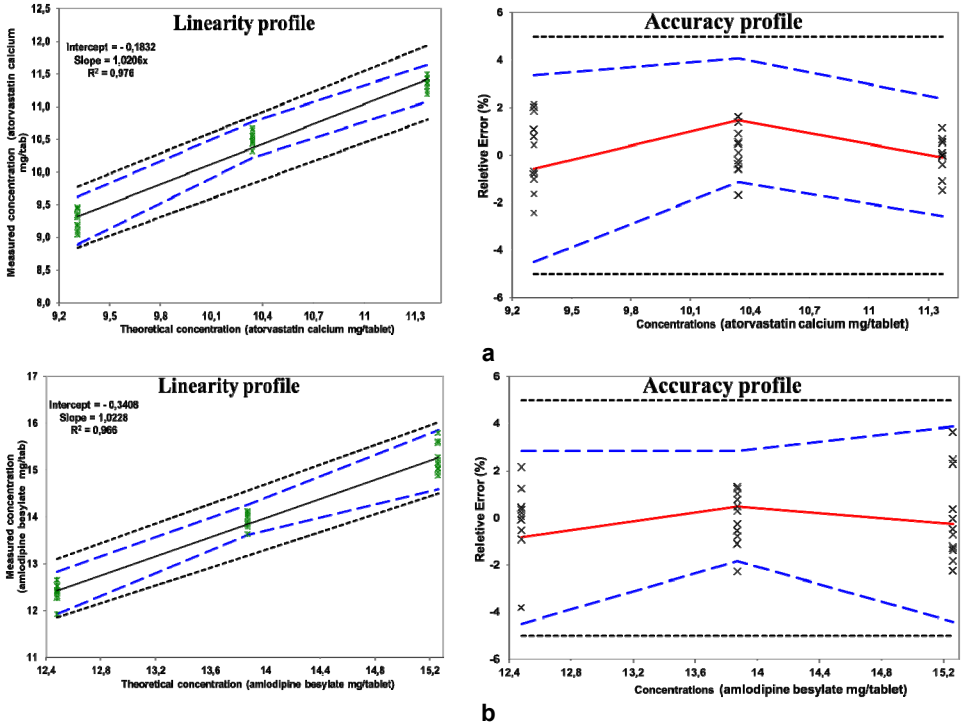

Figure 2. The linearity profiles (left) and accuracy profiles (right) the obtained for the NIRS method of simultaneous quantification of atorvastatincalcium (a) and amlodipine besilate (b).

According to the data presented above in Table 2 and Figure 2, the NIRS method using model (f, SNV-4) for atorvastatincalcium (standard normal variate pre-processing, 7 PLS factors and 4 spectral regions: 10000-8270; 7700-7120; 6800-5616; 5400-4243cm^{-1}) and model (l, FD+SNV) for amlodipine besilate (first derivative followed by standard normal variate pre-processing, 7 PLS factors and 1 spectral region: 10000-4200cm−1) is reproducible, accurate and linear (has an accuracy profile and a linearity profile within the acceptance limits set at ±5%,). So, it can be concluded that the NIRS method is linear, sufficiently precise and accurate for the direct (without any sample preparation) and simultaneous quantification of both APIs (atorvastatin and amlodipine) in tablets.

Application of the method

The results presented in the validation section indicated that the method could be used for the direct and simultaneous determination of atorvastatin and amlodipine content in tablets with active content 10 mg APIs/table (over the range of 9.31-11.37 mg/tablet for atorvastatin as atorvastatin calcium and over the range of 12.48-15.26 for amlodipine as amlodipine besylate). The NIRS method has been applied for the simultaneous quantification of both APIs in 4 control samples of tablet batches containing 10 mg APIs/tablet, strength which is the expected in the tablets available on the market. A reference HPLC method has also been used for APIs assay in the same control samples. The results obtained with the NIRS method and reference HPLC methods are shown in Table 3.

Table 3. Results obtained on control samples by NIRS method and HPLC reference method

	Atorvastatin			Amlodipine		
Control samples	HPLC*	NIRS	Recovery** (%)	HPLC*	NIRS	Recovery** (%)
P1	10.22	10.64	104.15	13.91	13.63	97.95
P2	10.29	10.45	101.56	13.81	14.01	101.43
P3	10.34	10.51	101.65	13.54	13.90	102.71
P4	10.60	10.43	98.39	13.80	14.11	102.24
Mean	10.36	10.51	101.44	13.77	13.91	101.08
SD		0.09			0.21	
t_{exp}		1.519			1.133	
P (type 1 error)		0,179			0.301	

*HPLC reference method
** Calculated as 100 ×NIR/HPLC

The NIRS predicted values for APIs content in control samples were compared with values obtained by the reference HPLC method, in terms of active content recovery, and the Student t test has been used for comparison of the methods. As presented in Table 3, similar results were obtained by both methods (NIRS and reference HPLC method). The average recovery was 101.44 for atorvastatin and 101.08 for amlodipine. The results did not show any statistical difference ($p>0.05$) between the results obtained using NIRS method and results obtained using the reference HPLC method.

CONCLUSIONS

In this work a NIRS method was explored for the direct, fast, non-destructive and non-invasive quantitative analysis of two APIs in a fixed-dose combination tablet. The two components were determined simultaneously using pre-processed spectra (standard normal variate, first derivative followed by standard normal variate) together with PLS multivariate calibration. The method was validated in terms of trueness, precision and accuracy, for active contents of 90-100-110%. The validation shows good statistical results and furthermore, application of the method and on real samples similar with the tablets on the marked proved that results obtained with NIRS are similar with those obtained by HPLC, used as reference method.

According to the data presented in this paper, NIRS based on PLS multivariate calibration could to be a suitable tool for non-destructive, direct and simultaneous prediction of the chemical composition of a fixed-dose combination that includes two active pharmaceutical ingredients (atorvastatin and amlodipine) combined in a single dosage form and is helpful in achieving the goals of Process Analytical Technology (PAT).

EXPERIMENTAL SECTION

Materials

Atorvastatin calcium (Hetero, India), amlodipine besylate (Hetero, India), microcrystalline cellulose (JRS Pharma, Germany), calcium carbonate (SPI Pharma, France), sodium croscarmellose (JRS Pharma, Germany), corn starch (Colorcon, UK) silicon dioxide (RohmPharma Polymers, Germany), magnesium stearate (Union Derivan, Germany).

Sample preparation for NIR analysis

A protocol was followed for calibration and validation, in order to develop and validate a robust NIRS method for the simultaneous quantification of two APIs. The protocol included batches and days as sources of variability. A training calibration set consisting in 26 different formulations of tablets containing different amounts of atorvastatincalcium and amlodipine besilate was prepared according to a D-optimal experimental design with 2 variables and 5 levels generate by Modde 10 software (Umetrics, Sweden) (Table 4).

Table 4. Composition of calibration/validation set according to an D-optimal experimental design

Exp Name	X_1 mg/tablet	X_2 mg/tablet	Exp Name	X_1 mg/tablet	X_2 mg/tablet
N1	8,27	11,09	N14	11,37	13,87
N2	9,31	11,09	N15	12,41	13,87
N3	10,34	11,09	N16	8,27	15,26
N4	11,37	11,09	N17	9,31	15,26
N5	12,41	11,09	N18	10,34	15,26
N6	8,27	12,48	**N19***	11,37	15,26
N7*	**9,31**	**12,48**	N20	12,41	15,26
N8	10,34	12,48	N21	8,27	16,64
N9	11,37	12,48	N22	9,31	16,64
N10	12,41	12,48	N23	10,34	16,64
N11	8,27	13,87	N24	11,37	16,64
N12	9,31	13,87	N25	12,41	16,64
N13*	10,34	13,87	N26	12,41	16,64

X_1– atorvastatincalcium, X_2 - amlodipinebesilate * - validation samples

Table 5. Qualitative and quantitative composition of calibration and validation samples

Concentration level	1 [a] 80%	2 [a,b] 90%	3 [a,b] 100%	4 [a,b] 110%	5 [a] 120%
Tablets composition (mg/tablet)					
Atorvastatin calcium	**8.27**	**9.31**	**10.34**	**11.37**	**12.41**
Amlodipine besylate	**11.09**	**12.48**	**13.87**	**15.26**	**16.64**
Microcrystalline Cellulose	78.26	75.84	73.42	71.00	68.58
Calcium carbonate	30.00	30.00	30.00	30.00	30.00
Croscarmellose sodium	6.00	6.00	6.00	6.00	6.00
Corn starch	15.00	15.00	15.00	15.00	15.00
Silicon dioxide	0.38	0.38	0.38	0.38	0.38
Magnesium stearate	1.00	1.00	1.00	1.00	1.00
	150.0	**150.0**	**150.0**	**150.0**	**150.0**

[a] calibration samples for API assay; [b] validation samples for API assay;

In the tablets the amount of APIs was between 8.27 – 12.41 mg/tablet for atorvastatin calcium and between 11.09–16.64 mg/tablet for amlodipine, respectively. This amount results from the preparation of atorvastatincalcium and amlodipine tablets with 10mg of each API/tablet and 150 mg tablet weight. The amount of API/tablet was 8.27, 9.31, 10.34, 11.37, 12.41 mg atorvastatin calcium and 11.09, 12.48, 13.87, 15.26, 16.64 mg amlodipine besilate respectively, corresponding to 80, 90, 100, 110 and 120% API content in the formulations (Table 5).

Tablets were prepared by direct compression. In detail, atorvastatincalcium, amlodipinebesilate, microcrystalline cellulose, calcium carbonate, sodium croscarmellose, corn starch and silicon dioxide were mixed using a planetary mixer (PRS type, Erweka, Germany) for 5 min. The powder blend for tableting was passed through the 0.8 mm sieve and remixed for 3 minutes in the same mixer. Subsequently, magnesium stearate was added to the mixture and mixed for 1 minute. A total of 150 mg of powder for tableting was filled in a die (Ø 7mm) and compressed using an eccentric tablet press (Riva, UK).

NIR analysis

NIR spectra were recorded using a Fourier-transform NIRS analyser (Antaris, TermoElectron, SUA) in Reflectance Sampling configuration. Each reflectance spectrum was acquired via OMNIC software (Termo Scientific, USA) by integrating 32 scans taken over a wave number between 4000cm^{-1} to 10000cm^{-1} with 8cm^{-1} resolution. Twenty different NIR measurements on twenty different tablets of the same batch tablets sample were recorded.

Model calibration

For the development of calibration models the PLS (Partial Least Squares) regression method from the OPUS Quant 6.5 (Bruker Optics, Germany) was used. Different pre-processing methods were applied in combination with the whole spectra or different spectral regions in order to find models with high predictive ability [19]. The predictive ability of a model was evaluated according to the following classical criteria: RMSECV (root mean square error of cross-validation), high correlation coefficient (R^2), low number of PLS factors and low bias [20,21]. The optimal numbers of factors for PLS were determined by a cross-validation procedure with groups of two spectra (each side of the sample being represented by a spectrum) [22].

Method validation

For validation, external independent sets of samples are needed. In order to validate the NIRS methods, the formulations from calibration sets corresponding to 90, 100 and 110% APIs content, (formulations N7, N13 and N19) were prepared using the same methodology presented previously. Four replicates for each concentration level were prepared in three different days as validation samples. There are several validation parameters that must be determined in order to be consistent with the recommendations of International Conference of Harmonization (ICH) and other regulatory (EMA, FDA) guidelines: accuracy, precision (repeatability and intermediate precision), linearity and range of application. The validation was performed according to the strategy proposed by Hubert *et al* [23] with slight modification according with a recent review on NIRS methods validation [24]. Calculation of the validation parameters (trueness, precision, accuracy) was performed in Microsoft Office Excel 2010 (Microsoft Corporation, USA)

Reference methods

Atorvastatin and amlodipine assay in tablets were performed using a reference HPLC-UV validated method. The chromatographic parameters were: column Phenomenex Luna C18 (2) 150 x 4,6mm x5µm; mobile phase acetate buffer (0.025M, pH 4.5): acetonitrile in gradient (0-2min, 55:45v/v; 2-5min 75:25vv); flow rate of 1.5 ml/min. The detection was performed at 236nm for atorvastatin and 246nm for amlodipine. Under the given chromatographic conditions the retention time was 2.01 minutes for atorvastatin and 4.01 minutes for amlodipine.

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