QUANTITATIVE CHARACTERIZATION OF POWDER BLENDS FOR TABLETS WITH INDAPAMIDE BY NEAR-INFRARED SPECTROSCOPY AND CHEMOMETRY

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Abstract

A NIR-chemometric method, that is able to directly quantify the active pharmaceutical ingredient (API) and two major excipients in pharmaceutical powder blends for manufacturing indapamide sustained release (SR) tablets, was developed and fully validated.

In order to develop calibration models for the assay of indapamide, hydroxypropylmehylcelulose (HPMC) and lactose, the NIR spectra of 25 series of powder blends (prepared according to an experimental design) were recorded. Further, they were analyzed by testing different pre-processing methods and using partial least-square regression (PLS). Using the best calibration models: Second Derivate (SD) for indapamide, First Derivate+Multiplicative Scatter Correction (FD+MSC) for HPMC and lactose, the methods were fully validated according to the ICH guidance. The validation results showed good precision, trueness and accuracy, between ±10% acceptance limits for the prediction of indapamide content and between ±5% acceptance limits for the prediction of lactose and HPMC content. Such quick NIR-chemometric methods require no sample preparation and successfully implement the Process Analytical Technology (PAT) concept in the manufacturing process of indapamide sustained release tablets.

Rezumat

S-a dezvoltat şi validat o metodă NIR-chemometrică pentru dozarea directă a substanţei active şi a doi excipienţi mai importanţi din amestecuri de pulberi destinate obţinerii comprimatelor de indapamid cu cedare susţinută.

Pentru a dezvolta modelele de calibrare pentru indapamid, hidroxipropilmetilceluloză (HPMC) şi lactoză s-au înregistrat şi s-au analizat prin metoda celor mai mici pătrate spectrele NIR a 25 serii de amestecuri de pulberi realizate conform unui plan experimental, testându-se diferite metode de pretratament. Utilizându-se cele mai bune modele de calibrare: *Second Derivate* (SD) pentru indapamid, *First Derivate+Multiplicative Scatter Correction* (FD+MSC) pentru HPMC şi lactoză, metodele au fost complet validate conform ghidurilor ICH. Rezultatele obținute la validare au arătat valori bune ale preciziei, exactității şi acurateţei, limitele de toleranţă fiind de ±10% pentru predicţia indapamidului şi ±5% pentru predicţia concentraţiei lactozei şi a HPMC. Astfel de metode NIR-chemometrice nu necesită prelucrarea prealabilă a probelor şi pot fi

implementate cu succes în cadrul conceptului Tehnologiei Analitice a Procesului la fabricarea comprimatelor de indapamid cu cedare susţinută.

Keywords: near infrared spectroscopy, chemometrics, indapamide assay

Introduction

The framework implemented by FDA in 2004 encourages the voluntary development of innovative pharmaceutical technologies based on PAT, a process-oriented vision, aiming to achieve highly efficient and reliable manufacturing processes [1]. In the field of quantitative analysis, HPLC has proven its value offering good selectivity, specificity and linear range. However, it requires lengthy method development, mixing of buffers and the disposal of volatile solvents for separation [2]. For these reasons analysis sessions are currently done off-line and take days.

The near-infrared (NIR) spectrum of a sample reflects both chemical and physical information [3] and is easy to be obtained, but it is also broad and non-selective compared to other analytical techniques (such as infrared or Raman spectra). The selectivity of the analytical method is provided by associating carefully developed multivariate calibration methods which ensures the quantitation of specific compounds in the presence of heavy interference given by other analytes in complex matrices, like, for instance, a pharmaceutical powder blend for tableting [4-8]. Chemometric interpretation of NIR spectra succeeds to accomplish the PAT initiative by providing quantitative information without prior sample pretreatment or separation, making this fast analytical technique ideal for at-line/online determinations [9].

The aim of this paper was to develop and validate such a NIR – chemometric method, able to directly quantify the indapamide, HPMC and lactose in powder blends for tableting without any sample preparation.

Materials and Methods

Materials: Indapamide (PharmaZell, Germany), Lactose monohydrate - Tabletose 80 (Meggle, Germany) Hydroxypropyl Methycellulose(HPMC)-Methocel K15M (Colorcon, UK), Aerosil 200 (RohmPharma Polymers, Germany), Magnesium stearate – Emprove (Merck, Germany).

Preparation of powder blends for NIR calibration. Ensuring an appropriate calibration set is an important issue in quantitative NIR spectroscopy applications. The spectra to be included in the library should contain every possible source of variability to guarantee the method's

robustness [3]. Therefore, an orthogonal experimental design with 3 factors (the three substances) and 5 levels (5 concentrations ranging between 80% and 120%) was used to generate the calibration set (Table I). An overall of 25 samples for calibration was prepared (Table II).

Table I

Experimental design factors and levels of variation

Substances/levels of variation	80%	90%	100%	110%	120%
INDAPAMIDE	0.571	0.643	0.714	0.786	0.857
Hydroxypropyl Methycellulose	28.01	31.51	35.01	38.514	42.014
Lactose monohydrate	55.879	59.450	63.026	66.598	70.169

Table II

Experimental design matrix for calibration set

Sample	X1	X2	X3	Sample	X1	X2	X3
N1	0.571	28.010	70.169	**N14**	0.786	35.010	62.955
N2	0.643	28.010	70.098	**N15**	0.857	35.010	62.883
N3	0.714	28.010	70.026	**N16**	0.571	38.514	59.664
N4	0.786	28.010	69.955	**N17**	0.643	38.514	59.593
N5	0.857	28.010	69.883	**N18**	0.714	38.514	59.521
N6	0.571	31.510	66.669	**N19**	**0.786**	**38.514**	**59.450**
N7	**0.643**	**31.510**	**66.598**	**N20**	0.857	38.514	59.379
N8	0.714	31.510	66.526	**N21**	0.571	42.014	56.164
N9	0.786	31.510	66.455	**N22**	0.643	42.014	56.093
N10	0.857	31.510	66.383	**N23**	0.714	42.014	56.021
N11	0.571	35.010	63.169	**N24**	0.786	42.014	55.950
N12	0.643	35.010	63.098	**N25**	0.857	42.014	55.879
N13	**0.714**	**35.010**	**63.026**				

The indapamide and 10% of the quantity of lactose were homogenized using a planetary mixer (PRS type, Erweka, Germany) for 10 minutes and then passed through the 0.600 mm sieve. This mixture was then homogenized with the remaining lactose, HPMC and Aerosil for 10 minutes. Further, magnesium stearate was added and the mixing continued for another minute.

NIR analysis of the powder blend. 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 by integrating 32 scans taken over a wave number between 4000 cm^{-1} to 10,000cm^{-1} with 8cm^{-1} resolution.

NIR spectra processing. The development of a calibration model consisted in checking different spectral pre-treatments, as well as their combination with different spectral ranges containing strong bands of indapamide. Multivariate calibration was then applied to chemometric approaches based on PLS (Partial Least Squares) regression using Opus Quant (Bruker Optics, Germany) software. This software allows models' validation via "full cross-validation". In this procedure, iterative calibrations were performed by removing in turn each standard from the training set and then predicting the excluded sample with that calibration [10]. The spectral pretreatments tested with the aim to build the calibration models included straight line subtraction (SLS), vector normalization (SNV), min-max normalization (MMN), multiplicative scatter correction (MSC), first derivate (FD), FD+SLS, FD+SVN and FD+MSC.

Method validation. Once a calibration is developed and favourable predictions are expected, the method has to be validated in order to be accepted for routine use. For external validation independent sets of samples are needed. For this purpose, four sets of N7, N13 and N19 formulations were prepared using the same technique. The validation was performed according to the strategy proposed by Hubert et al [11-14].

Results and Discussion

Models calibration development

The choice of the adequate number of factors (main components) and spectral data pre-treatment model is critical in a PLS chemometric calibration. This is important in order to avoid the "over-fitting" phenomenon. Different methods were suggested to achieve this [7, 8]. The selection of the optimal number of factors was performed using the Haaland and Thomas criteria [9]. Also, the selected spectral pre-treatment model is the one with the lowest number of factors and whose RMSECV (Root Mean Square Error of Cross Validation) is not significantly higher than the RMSECV of the model with one more factor. For each pre-processing method, the squared correlation coefficient, R^2, between actual known concentration and predicted concentration, was determined, in order to evaluate the predictive ability of the model. The results obtained during the method development are presented in Table III. Figure 1 shows the RMSECV plotted as a function of PLS factors, for the quantification of (a) indapamide, (b) HPMC and (c) lactose in powder blends for tableting with different spectra pre-processing methods.

Table III

Statistical parameters and numbers of the principal components in the PLS method for the quantification of **indapamide**

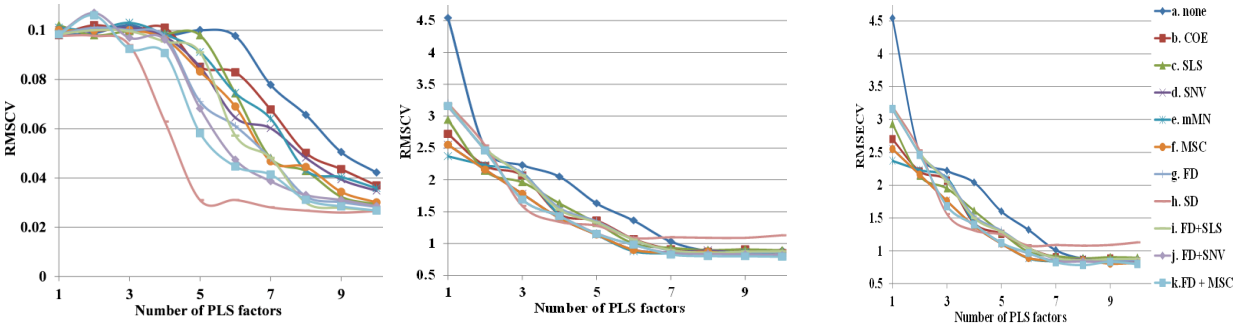
Model	a	b	c	d	g	**h**	**I**	k
Pre-treatment	None	COE	SLS	SNV	FD	**SD**	FS+SLS	FD+MSC
Spectral range selected	9000-8338; 7500-7996;6100-5570;5353-4730; 4501-4000 (cm^{-1})							
Nr. of PLS factors	10	10	10	10	9	**8**	9	9
R^2	80.74	85.25	90.76	86.89	90.13	**92.23**	91.57	91.2
RMSECV	0.422	0.037	0.0293	0.0348	0.0302	**0.0268**	0.0279	0.0285
Bias	0.00018	0.00033	-0.00074	0.00058	0.000164	**-0.00048**	-0.0013	-0.000135

for the quantification of **HPMC**

Model	a	b	e	f	g	H	j	k
Pre-treatment	None	COE	mMN	MSC	FD	SD	FD+SVN	**FD+MSC**
Spectral range selected	8836.4-4000(cm^{-1})							
Nr of PLS factors	8	7	6	7	7	6	6	**7**
R^2	96.74	96.58	97.02	96.96	96.74	95.11	97.04	**97.03**
RMSECV	0.887	0.91	0.848	0.857	0.888	1.09	0.846	**0.827**
Bias	-0.00346	-0.0142	-0.0122	-0.00957	-0.0082	0.0093	-0.058	**-0.00815**

for the quantification of **lactose**

Model	a	b	b	e	f	g	h	**k**
Pre-treatment	None	COE	SNV	mMN	MSC	FD	SD	**FD+MSC**
Spectral range selected	8836.4-4000(cm^{-1})							
Number of PLS factors	8	7	6	7	7	7	6	**7**
R2	96.78	96.64	96.7	97.08	97	96.77	95.13	**97.19**
RMSECV	0.883	0.901	0.893	0.841	0.0852	0.883	1.08	**0.825**
Bias	0.00357	0.014	0.0195	0.0121	0.0095	0.0077	-0.00984	**0.00689**



a) b) c)

Figure 1

Root Mean Square Error Of The Cross Validation (RMSECV) variation depending on the PLS factors for the quantification of (a)indapamide, (b)HPMC and (c)lactose

For the assay of indapamide, the lowest number of PLS factors (8), the highest correlation factor (R2=92.23) and the best capacity of prediction (lowest RMSECV) were obtained at the same time by Second Derivation. Therefore, the (h) model was further used for the method validation. The (k) model, namely FD+MSC, was chosen as the best fitted model for the quantification of HPMC and lactose because it showed better predictability (lower RMSECV) than the ones with the minimum number of PLS factors(6).

Methods validation

The validation protocol was realized according to ICH Q2 (R1) guideline requirements [15]. The validation was performed according to the strategy proposed by Hubert *et al*. [7,8,9]. This approach used tolerance intervals as statistical methodology that allows predicting a region of concentration where each future result has a probability to fall defined by the analyst [16]. Table IV shows the validation criteria of the developed method.

Table IV

Validation results of the NIR-chemometric methods

for the quantification of **indapamide**

Concentration level (% indapamide)	Trueness		Precision		Accuracy	
	Relative bias (%)	Recovery (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	Relative tolerance limits (%)	Tolerance limits (µg/mL)
0.643	0.564	100.56	3.37	2.94	[-7.62, 8.75]	[0.594, 0.699]
0.714	0.814	100.81	2.17	2.01	[-4.90, 6.53]	[0.679, 0.761]
0.786	-1.304	98.70	1.93	1.71	[-6.10, 3.49]	[0.738, 0.813]

for the quantification of **HPMC**

Concentration level (%HPMC)	Trueness		Precision		Accuracy	
	Relative bias (%)	Recovery (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	Relative tolerance limits (%)	Tolerance limits (µg/mL)
31.51	0.422	100.42	1.81	1.59	[-4.01, 4.85]	[30.24, 33.05]
35.01	0.432	100.43	1.00	1.01	[-2.51, 3.38]	[34.12, 36.19]
38.51	-1.067	98.93	1.07	1.09	[-4.23, 2.10]	[36.89, 39.31]

for the quantification of **lactose**

Concentration level (%lactose)	Trueness		Precision		Accuracy	
	Relative bias (%)	Recovery (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	Relative tolerance limits (%)	Tolerance limits (µg/ml)
59.45	0.6706	100.67	1.12	1.19	[-2.8, 4.2]	[57.77, 61.93]
63.03	0.0728	100.07	1.17	1.09	[-3.0, 3.2]	[61.12, 65.02]
66.06	-0.0068	99.99	1.01	1.09	[-3.2, 3.2]	[64.45, 68.73]

The trueness of the method was evaluated by calculating the recovery and the relative bias. The precision of the method was assessed by calculating two parameters: repeatability and intermediate precision.

The **indapamide** assay showed good recovery (close to 100%) for all three levels of concentration. The best repeatability and intermediate precision values were obtained at the highest indapamide content in powder blends, 0.786%, while the largest relative tolerance limits, [-7.62, 8.75], were obtained at the lowest indapamide content, 0.643%.

The **HPMC** assay achieved good trueness and precision with the following maximum values: -1.067 % relative bias for the lowest HPMC content, 1.81% repeatability and 1.59% intermediate precision for the highest HPMC content. The relative tolerance limits do not exceed ±5% for any level of concentration.

The **lactose** validation values seem to be the most precise and accurate. For the upper level of concentration, 66,06%, the method achieved 99.99% recovery, -0.0068% relative bias and [-3.2, 3.2] relative tolerance limits. Even for the lowest concentration level, the quantitation of lactose falls within a narrow range of relative tolerance: [-2.8, 4.2].

The linearity and accuracy profiles of the prediction models are shown in Figure 2. The linearity profile was represented by plotting the measured concentrations of the external validation samples according to the theoretical concentrations while the accuracy profile illustrates the relative error of the model. The dashed limits on the graphs correspond to the accuracy profile and the dotted curves represent the acceptance limits at ±10% for indapamide and ±5% for HPMC and lactose, expressed in concentration units.

According to Table IV and Figure 2, the accuracy of the method for the entire concentration range (80-120%) is adequate and the most accurate values were obtained for the medium concentration level (100%) of all three substances.

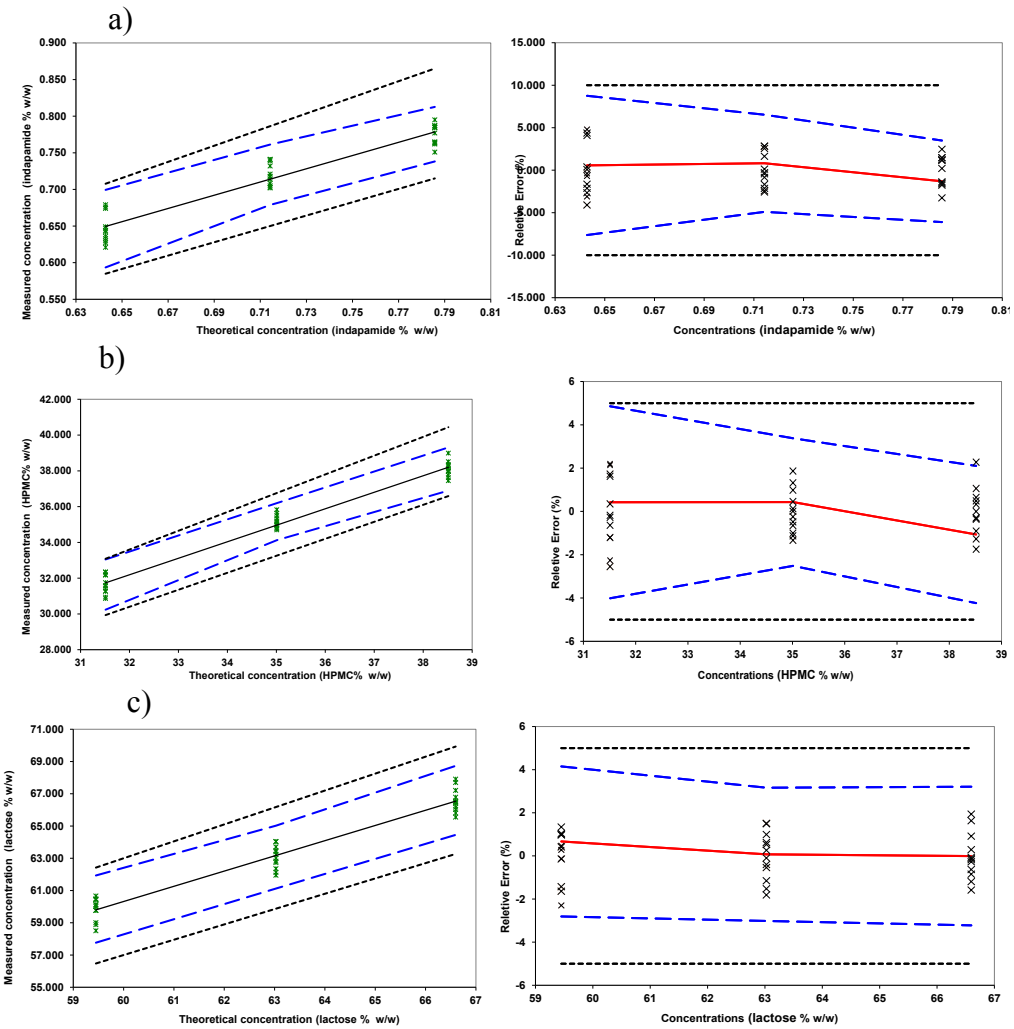


Figure 2.
The linearity profiles (left) and the accuracy profiles (right) obtained for the NIR-chemometric methods of quantification of: (a) indapamide, (b)HPMC and (c)lactose

Conclusions

NIR-chemometrics methods were developed for the direct quantification of indapamide (API) and two major excipients (HPMC and lactose) in powder blends for tableting. To achieve this, different types of pre-processing techniques were tested. Further, using the most predictive calibration model the method was fully validated according to the ICH guidance. The validation results showed good precision, trueness and

linearity for the determination of indapamide, HPMC and lactose in powder blends for tableting with contents ranging from 80 to 120% substance content. As far as accuracy is concerned, the method fell between ±10% acceptance limits for the prediction of indapamide content and between ±5% acceptance limits for the prediction of lactose and HPMC content.

The developed methods allow direct quantification of the three subtances using only one NIR spectrum of the powder and require no sample preparation. Such quick NIR – chemometric methods can be used for in line/at line monitoring of the manufacturing process of indapamide SR tablets and are helpful in achieving the goals of the PAT concept.

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