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DETERMINATION OF API CONTENT IN A PILOT-SCALE BLENDING BY NEAR-INFRARED SPECTROSCOPY AS A FIRST STEP METHOD TO PROCESS LINE IMPLEMENTATION

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Abstract: Near infrared (NIR) spectroscopy was used for estimation of powder blend homogeneity and manufacturing control of a medicinal product powder mixture containing active pharmaceutical ingredient (API). Aiming at initiating a Process Analytical Technology (PAT) activity, the first step was a stationary mode *at-line* evaluation. In this, the content of pharmaceutical active compound in the powder mixtures intended to the direct tabletting was estimated based on recorded NIR spectra. Five formulations containing different quantities of API were prepared and analyzed also by a reference method – UV-Vis spectroscopy. A chemometric model was developed for calculation of the API amount in the mixtures. The Principal Component Regression (PCR) and Partial Least Squares (PLS) algorithms were used to obtain a model useful in further implementation for the PAT recommendations, into *in-line* blending control.

Keywords: mixing, powders, pharmaceuticals, particulate processes, near infrared spectroscopy

Process Analytical Technology (PAT) is based on quality risk management by identification of the main risk elements and their influence on the final product. Priority activities are focused on minimizing undesirable events in the manufacturing process and avoiding defects in the resulting products (1). PAT causes shortening of the production cycle *via on-line* analysis during the process, what results in reduction of energy and raw materials used (2).

Implementation of the PAT requires that the critical process parameters are identified for each technological process, and the choice of suitable analytical technique. An appropriate technique that is often used in quality systems is near infrared spectroscopy (2-5), which is suitable for both *on-, in-* and *at-line* examinations. An additional advantage of the NIR is the possibility to handle and control a large group of industrial/technological variables that need to be optimized in manufacturing processes (6). The unique properties of the NIR spectroscopy method make it suitable for monitoring of many manufacturing processes (7), such as fluidized-bed granulation (wet, *in-line*), mixing, spraying and drying (8) in order to determine the end-point of those processes (4). Using NIR, the relationship between the amorphous and crystalline forms of active pharmaceutical ingredients (APIs) can be determined with a lower detection limit (5, 9), than with other methods. With NIR spectroscopy in the reflectance mode, it is also possible to determine the thickness of the coating (10-12). Many parameters of the medicinal products are also commonly controlled with NIR spectroscopy, e.g., hardness and porosity (13), size, compression strength (14), and disintegration time (4, 5, 15). The use of a chemometric model with the NIR spectroscopy analysis with Chemical Imaging Option reduces the number of samples needed to develop an operational system (16). Developing a chemometric model is usually time consuming and expensive, and there have been several attempts to create a universal calibration model, however, in accordance with established standards, one has to develop a unique model for each new process (4, 17, 18).

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Table 1. Applications of NIR spectroscopy in pharmaceutical processes connected with blending/mixing.

Composition	API content [%]	Wavelength [nm]	Blending time [min]	RPM [rpm/min]	Chemometric model[1]	Reference method	Aim of the work	Determination (References)
Salicylic acid, lactose	90	1100–2200	50	12.8 20.3	PLS, SIMCA PC-MBEST PCA PCR	UV/Vis	Humidity impact study (RH = 20 and 60%), API content (3, 7, 11%), blending rate and density effect on homogeneity. Comparing two different algorithms for the blending process. Development of a quantitative model for determination of mixture homogeneity, humidity impact, blending rate and API content.	Quality (4, 19) Quantity (20)
Paracetamol, Avicel, Lactose	70	1400–1675	0.5; 1; 2; 5; 10; 15; 20; 24; 30 and 40	25	PCA PLS	UV/Vis	Distribution of mixture components and simulated chemical concentrations of samples in small scale.	Quality and quantity (16)
Otilonium bromium, microcrystalline cellulose, potato starch, carboxymethyl starch	*	1100–2500	25	*	MSD	Average of last 5 measure-ments of blending samples	Monitor blending by NIRS in order to ensure uniformity in a mixture consisting of three typical pharmaceutical excipients.	Quality (21)
Salicylic acid, lactose	90	1100–2500	30	23	PCA	UV/Vis	Impact of the location and number of measurement points needed to achieve a good distribution of the ingredients on the end-point determination.	Quality and quantity (22)
Gemfibrozil, microcrystalline cellulose, pregelatinized starch	*	1100 ± 2200	60	*	PLS	UV/Vis	Analytical control of different pharmaceutical production steps, involving various types of samples (blended products, cores and coated tablets), with a method based on a common chemometric model.	Quality and quantity (23)
Chlorpheniramine maleate, lactose, magnesium stearate, microcrystalline cellulose	60	980–2100	30	10	PLS	*	Effects of premixing on the result of NIR analysis for quantification of drug and excipients in the blending process.	(24)
Paracetamol, Avicel, lactose, potato starch	80	911–1680	90	*	PLS	*	Blending behavior at the location of the two sensors of a NIR spectrometer, and the impact on the end-point.	(25)
API, Crospovidone, microcrystalline cellulose, lactose	*	1350–1500 1500–1800	8, 20	25, 10	PLS	HPLC with UV/Vis detection	Transfer of the calibration model from laboratory to industrial scale.	(26)

Table 1. cont.

Composition	API content [%]	Wavelength [nm]	Blending time [min]	RPM [rpm/min]	Chemometric model[1]	Reference method	Aim of the work	Determination (References)
Hydrochlorothiazid, lactose, sodium carmelose, magnesium stearate	90	1200–2400	1; 5; 10; 15; 20; 30	25	PCA MBEST χ^2 test	UV/Vis	An approach to real-time determination of blend homogeneity using NIRS.	(27)
Paracetamol, microcrystalline cellulose, colloida silicon dioxide, magnesium stearate	10-15	1130–1650	0.5, 1, 1.5, 2	1000	PLS	AutoCal Off-line calibration	Real time monitoring of the continuous mixing process.	(28)

[1]PLS – partial least squares, SIMCA – soft independent modelling of class analogy, PC-MBEST – PC-Modified Bootstrap Adjusted Single Sample Technique, PCA – Principal component analysis, PCR – Principal components regression, * no data available.

In the last decade, many publications have presented results of tablets' mixture homogeneity determinations using near infrared spectroscopy. Examples are e.g., determination of the end for the mixing process, and the influence of environmental factors, such as humidity, number of revolutions per minute, concentration of API and other ingredients on the manufacturing process (Tab. 1). Other authors have determined the locations and number of measurement points needed to achieve a good distribution of the ingredients during the mixing process. Numerous studies have also focused on chemometric model that the best describes the collected data, calibration of methods, and scaling up.

A review of current literature shows that NIR is an excellent alternative to UV/Vis and HPLC as a method for assessing the homogeneity of mixtures. With this technique, not only the concentration of the API, but also of the excipients is included. It has been confirmed that a homogeneous mixture of all components is important to ensure required quality (24-28). It is usually quick and easy to achieve a uniform mix of the API, while for the excipients an individual approach is required (24).

At the beginning, the realization of homogeneity estimation is almost often determination of API concentration of powders during mixing/blending. These processes occur in more than 70% of all the processes in pharmaceutical manufacture of medicinal products. Powder blending has predominantly been a batch process both in practice and research (29). It is a crucial process of pharmaceutical preparation development (16). Properly controlled, provides an ideal homogeneity with a proper end-point determination (24) what is simultaneously correlated with the good API distribution in a dosage form, high quality formulation and appropriate therapeutic effect at the end. However, to manufacture a completely homogeneous product, is also essential to know the behavior and concentration of the excipients, as has been shown in many studies (21). With methods based on NIR it is possible to control simultaneously all components of the mixture.

NIR spectroscopy can be used in industrial manufacturing processes, in a much larger scale than in the laboratory, as has been repeatedly confirmed in studies where the process operation has been validated using reference methods (30). It has been demonstrated that the off-line laboratory method can be scaled up more than fifteen times to an industrial scale, further underlining the reliability and accuracy of the new technology (26). PAT implementation acquires preliminary the small-scale testing of a method intended to be involved into the

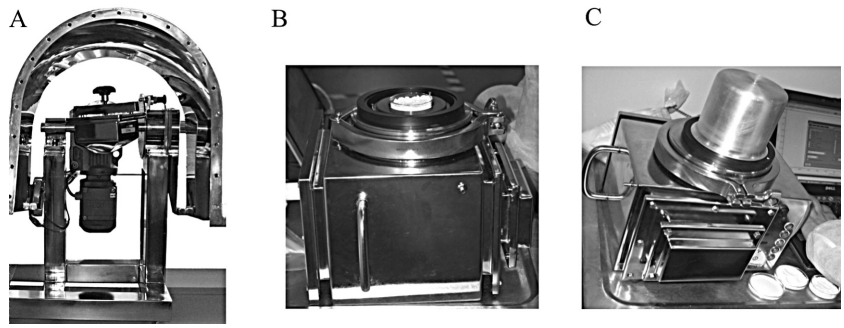

Figure 1. Powdered sample preparation, blending and spectra recording A) 1L conical laboratory mixer for powders; B) collected and poured samples on a Petri dish placed on the sapphire window of NIR spectrometer, C) covered sample and NIR spectrum being scanned

larger, production line (*in-*, *on-line*). Despite this, it is surprising that there are many researches describing *in-line* and *on-line* processes without earlier *at-line* scale recognition. Such works should enhance the *at-line* application of NIR spectroscopy and encourage further *on-line* application. This is the reason for presenting our results in a current form. A particular stage of a general production is blending of powders, therefore, homogeneity have significant impact on the end point achieving and also on the tabletting processes quality, lastly. This is the minor argument for our systematic presentation of the first-step of results in this work.

The aim of this study was to use the revolutionary miniature near-infrared analyzer, the Antaris™ Target, to predict blend uniformity on stationary mode in this work. Generally, there is a lack of *at-line*, preliminary studies of API determination in a powder mixture before tabletting. The same apparatus and model will be used in a case of production step of manufacturing process, blending in the next paper just being prepared.

Blending process is one of the most important processes and must be controlled effectively and very fast, therefore, NIR spectroscopy is popularly proposed method and according to our best knowledge still not implemented by many pharmaceutical manufacturers. Some descriptions of blending control had already been published, but this work is a starting material for the initiation of NIR implementation. Besides, there is a lack of such systematically presented work, from a pilot-scale to process line NIR spectroscopy method is recommended by Pharmacopoeias, but still being introduced in academic society and implemented into some industrial areas.

EXPERIMENTAL

Materials

A powdered EP grade API (Pharmaceutical Works "Polpharma", Poland) and the following excipients: pregelatinized corn starch (Starch 1500, Colorcon, USA), cellulose powder (Vitacel 70, JRS Germany) and microcrystalline cellulose (Vivapur PH 102, JRS Germany) were added proportionally to the weight of 454 g.

Blending and NIR spectra recording

Accurately weighted samples were thoroughly mixed in the conical laboratory mixer for powders (Kates Company, Poland) placed with 20 degrees inclination, and rotating at 12 rpm for 25 min. (Fig. 1 A). After that, a small amount of blended powder was collected and poured on a clean and dry Petri dish forming a thickness of about 5 mm by spatula (Fig. 1B). A layer of granules, mixed in the blender, with a thickness of about 5 mm was poured on a clean and dry Petri dish to record the near-infrared spectra (Fig. 1C). A revolutionary miniature near-infrared analyzer, the Antaris™ Target, was used in a case study to predict blend uniformity on a bin blender in a pharmaceutical manufacturing plant. The Antaris Target blend analyzer is a MEMS based NIR analyzer with a spectral range of 1350-1800 nm. It is powered by a semiconductor-based NIR tunable laser, and uses a high-resolution (1 nm) Fabry-Pérot tunable filter for wavelength selection. The unit is battery powered and uses an accelerometer-based triggering system to initiate data collection. Scan speed is approximately 100 milliseconds per scan. The unit has no moving parts and is totally insensitive to vibration. It attaches to the lid of the

bin blender easily, and uses a sapphire window in a modified bin lid for transmission of NIR energy onto the sample. The Antaris Target Blend Analyzer camera (Thermo Scientific Company, USA) was used for collecting data from a static samples with the analyzer in the benchtop (so called *at-line*). Using the Operation Result program the diffuse reflectance mode were directly set for powder probes.

Calibration and validation samples were scanned only from laboratory made samples in the static position of spectrometer.

Three spectra were recorded for each sample, along the wave number range of 4000-10,000 cm^{-1} from an average of 4 scans and with a 4 cm^{-1} resolution. The dish was placed on the measurement port (sapphire glass), covered and analyzed.

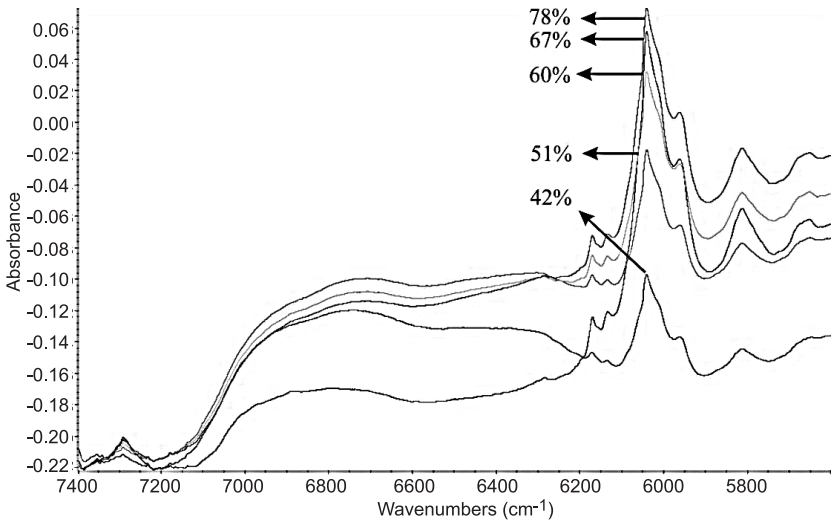

Figure 2. NIR spectra of formulations with different contents of the API

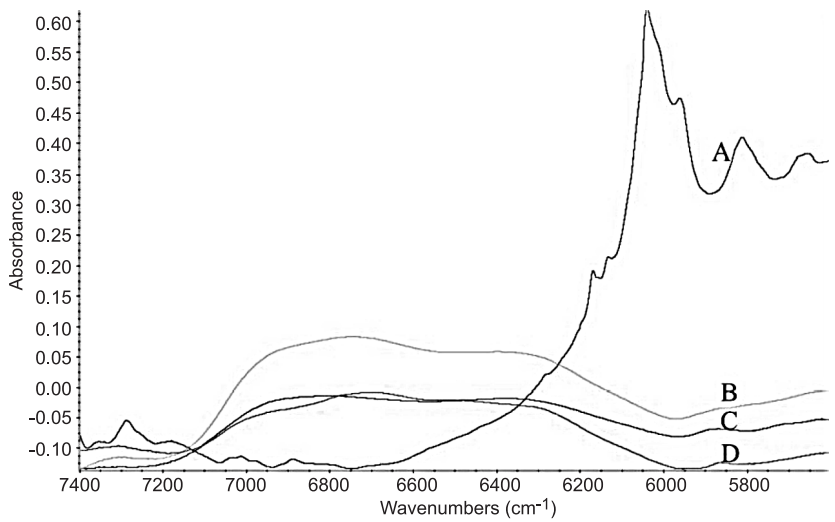

Figure 3. Raw NIR spectra of the individual blend components: A - API; B - powder cellulose; C - corn starch, D - microcrystalline cellulose

Preparation of samples

The samples used in calibration development were laboratory made samples. Laboratory samples were prepared from powders of variable amounts of the excipients and API in order to create an extended range of the target concentration 42, 51, 60, 67 and 78% (w/w). Mixtures with respective quantities of API, pregelatinized corn starch, cellulose powder and microcrystalline cellulose for each calibration sample were prepared in a batch size of 500 g. Five independent series consisting each API concentration were weighed and thoroughly mixed in a 1 L conical laboratory mixer for powders. All mixtures except those with a concentration of 60% (w/w), which was used as validation sample, were used as calibration samples, summarily 60 samples, because each sample was scanned three times. Validation samples, summarily 15 (five independently weighted and three times scanned) are dedicated to the original composition of drug product.

Validation of NIR calibration models

Two approaches were used to validate the respective calibration model. In the first one, the number of principal components (PC) required to minimize the root mean squared error of calibration (RMSEC) and root mean standard error of prediction (RMSEP) was carried out. In the second approach, 25 independent validation samples after blending (from randomly chosen serial number) were prepared and scanned using the same procedure to that of calibration samples. Prediction result using the respective calibration model was compared against the reference spectroscopic values.

Chemometric method

Based on the resulted NIR spectra, a method was created and verified using TQ Analyst 8.0.1.36, chemometric software. Principal component regression (PCR) and for comparison, partial least squares (PLS) were used as a two-step multivariate calibration method (31). Preliminary, a principal component analysis of the data matrix X was performed, and then the multiple linear regression (MLR) between scores obtained in the PCA step and the API concentration was modeled. For a pilot scale method performance of drug compound concentration determination this model appears to be sufficient (32).

Reference method

UV-Vis spectroscopy was used as the stated reference method for quantitative determination of API in the powder mixture for a tablet preparation. Spectroscopic measurements were made with the Lambda 40P spectrometer (Perkin Elmer Co.) from each homogeneity sample after blending, so that the average reference value for each concentration was determined from five analyzed samples. A 10 mm matched quartz cell was used for experiments. All used solvents were of analytical grade.

The same powder samples scanning by NIR spectrometer were used for UV-Vis method and API concentration determination. An independent portions of about 120 mg were used for analysis.

RESULTS AND DISCUSSION

The shape of the spectra of mixtures with different contents of the API is presented in Figure 2. Raw spectra of the individual components are shown in Figure 3.

Table 2. Method parameters.

Type of analysis	Quantity	Pre-processing	
Component analyzed	API	Data format	First derivative
Chemometric model	PCR	Wavelength range	6200–5700 cm^{-1}
Optical path length	Constant	Normalization	Centered

Table 3. Analysis of variations for six selected NIR spectra of the mixtures contain 51 and 67 % API.

	Sum of squares	Degree of freedom	Mean square	F ratio
Between standards	1.347	1	1.347	-
Between measurements	0.082	4	0.020	65.8

Generally, calibration samples were prepared by assigning the reference values obtained from laboratory method, to the powder blend. There is a possibility of some variations in the composition of random samples drawn from a powder mixture. Therefore, samples used for spectrum scanning were inserted in the program, and also the absorbance values of the different drug compound concentrations that were measured with the reference method (UV/Vis spectroscopy) were adjusted as representative.

A method was developed for determination of the percentage of drug compound quantity in a sample using PCR. For this, the wavelength range characteristic for API was used, i.e., 6200 - 5700 cm^{-1} (Tab. 2). In order to verify if the method can be used for a quantitative evaluation of the spectral data obtained from a powders with API, a feasibility test (F) was done to investigate if there were significant differences between the mean characteristics within a group and between groups of data. The test was done on six recorded NIR spectra: three from the first series of mixtures with 51% of API, and the first three recorded with 67% API (Fig. 4).

The sum of squares for the difference between measurements at a given level of API was small, but

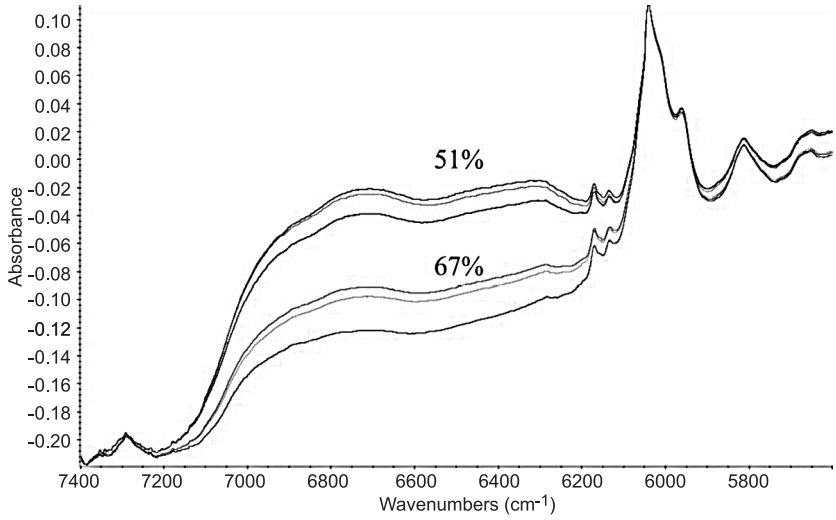

Figure 4. NIR spectra selected to perform the feasibility test.

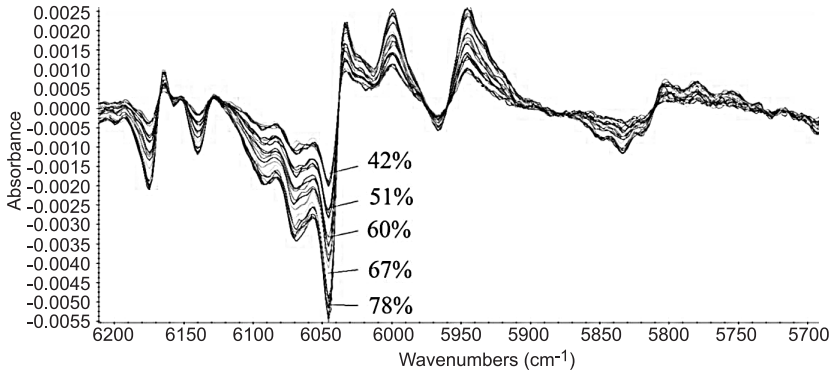

Figure 5. The first derivative of the NIR spectra used to develop a chemometric model for homogeneity detection in blending mixtures with drug compound

between the levels it exceeded 1 (Table 3). An F test was used to estimate the ratio of these two variances, and the result was much higher than 1, indicating that the samples differed significantly, and enough to continue developing a suitable method.

Next, the first derivative was calculated using 5 nm spacing in order to better observe the dependence of the absorbance on the drug compound concentrations (Fig. 5). For 42% and 51% concentrations, the transformed first derivative spectra were distinctly different. As the concentration percentage rose, the data clustering was less visible. This is due to a large distribution of the spectra representing 67% content. However, it is clear from any specific

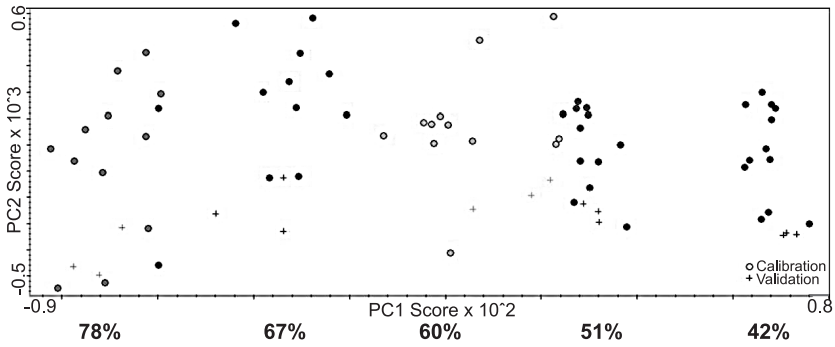

Figure 6. The distribution of individual data points in the main components of the principal component analysis, PC 2 *versus* PC 1

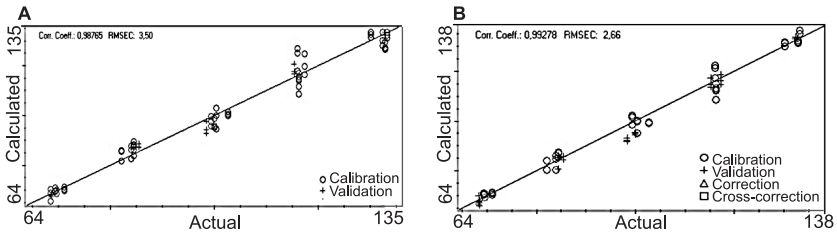

Figure 7. Relative difference between concentrations of API calculated from NIR spectra and measured with UV/Vis as a function of actual concentrations in a case of A - PCR algorithm and B - PLS algorithm

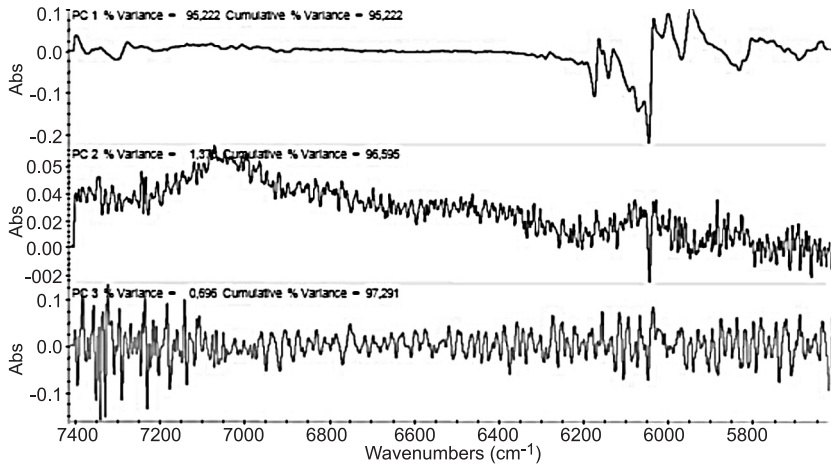

Figure 8. Spectra for the first three principal components generated by a chemometric method for spectra of API

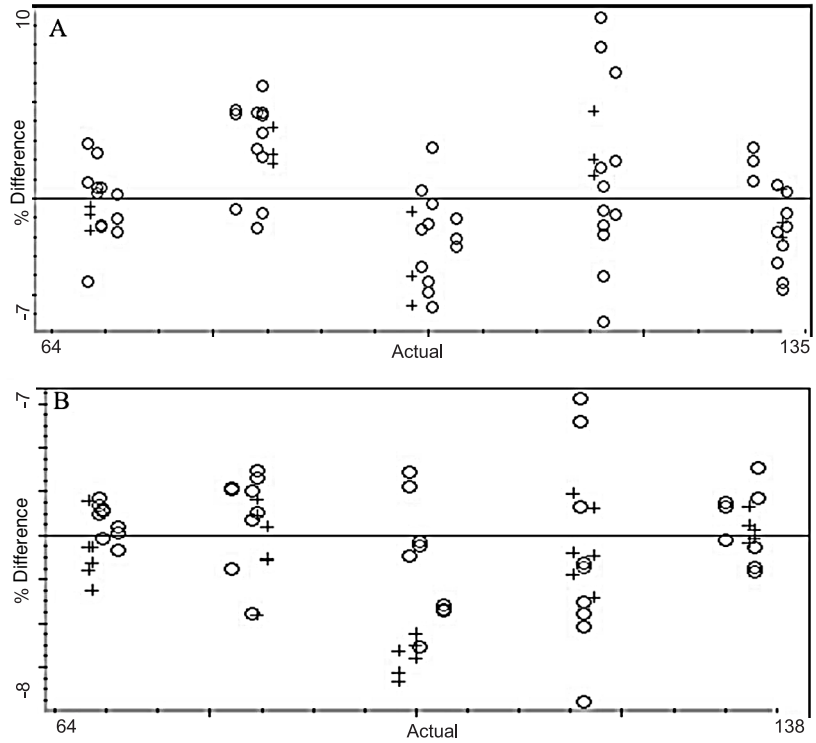

Figure 9. Relationship between the concentrations of API calculated from NIR spectra and measured with UV/Vis spectroscopy using principal components analysis using A - PCR algorithm and B - PLS algorithm

peak in the graph that there is a relationship between the increase in content of the active substance and the amplitude of the curve.

The thickness of the absorbing layer was assumed constant (i.e., constant path length). Data were normalized using a centering operation recommended for the principal components method (18). The transformation was performed on spectra used for calibration before the calibration of the method. It relies on calculating the mean of the individual spectra data points and subtracting it from each calibration spectrum. Spectra stored and analyzed with the method have not been modified.

The PCA analysis of the calibration matrix present that the first principal component separates the samples from the concentration mean (Fig. 6). The calibration approach based on the change in slope of the best fit line through the results from NIR spectra and reference method. The distribution of individual data points (for standards) in the first two principal components is shown in Figure 6. Distribution should be random in order to best describe the whole data set.

This was also the case for the model used (Fig. 6). The points are arranged on the OX axis, described by PC1, according to a decreasing content of the API. Distinctive groups can be distinguished although for API content level of 60.0 and 67.0% presented a large variation. Differences between the value of a concentration of API obtained from the reference analysis and the value estimated from the NIR method are presented in Figure 7. PLS model is more suitable to achieve smaller value calibration error (RMSEC 2.66) than in a case of PCR, 3.5.

The main components of the method were calculated from the first derivatives of the calibration spectra. Variations appear as peaks in the spectra, and the more distinct they are, the more useful information they contribute to the model. Noisy or shapeless spectra contain less significant information (Fig. 8).

Spectra were generated for the entire wavelength range, 7400-5600 cm^{-1}, and the first three components are shown in Figure 8. The spectrum of PC 1, carrying most of the information about the variability, had strongly marked and characteristic

peaks for drug compound in the range 6200-5700 cm^{-1}. For the other two components, the spectra were noisier, indicating a much lower amount of relevant information in them. The first component of the PCR described more than 95% of the accumulative variability of the whole spectral range (Full Spectrum Contribution) and 98% of the analyzed region.

PCR calibration model was evaluated and the RMSEC and the RMSEP were calculated and compared. The following prediction errors were: 3.50 and 2.70, respectively.

For comparison, prediction errors obtained by the usage of PLS were not appropriate 2.66 (RMSEC) and 2.83 (RMSEP) because of the higher value of prediction possibility. Nevertheless, they are very similar, so that this model might also be used to blending process implementation. From different options of pre-processing the number of latent variables for the minimum RMSEC was always higher than the number of latent variables for the minimum value of RMSEP.

API concentration correlation plots of RMSEC are presented in Figure 9. The results from the model developed for the NIR analysis of the API content in the mixture agreed well with the results from the reference method, with a 45% slope of the rectilinear relationship. The relative difference between the calculated concentrations and the reference values were randomly distributed when plotted against increasing concentrations, further confirming the linear relationship (Fig. 9). PLS model allowed to achieve less distribution of calibration and validation samples than PCR.

The mean of the calculated concentrations of API amounted to 97% of the reference values, what is observed for three component model. The method based on three components predicted the concentration of API equally well as the one with seven components. For the earlier recorded spectra used for validation, the calculated result was 95% with a relative standard deviation of 2.9%. It turned out that a change in external conditions during the measurement had a small effect on the concentration determination. The difference was within the acceptable error of 5%.

CONCLUSIONS

This laboratory scale study has resulted in a method for measuring the content of API in an experimental powder mixture. The working target of used miniature near-infrared analyzer, the Antaris Target is to predict blend uniformity on a bin blender in a pharmaceutical manufacturing plant. A pilot-scale testing of power mixtures presented in this work allow to apply such apparatus also for small scale process, even academic. NIR spectra of formulations with API with various contents demonstrated a wavelength range that was specific for drug compound. Different concentrations of API in the experimental samples resulted in different absorbance values. In order to improve the predictability of the method, we intend to extend the study to production-scale samples.

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