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The application of Near Infrared Reflectance Spectroscopy (NIRS) for the quantitative analysis of hydrocortisone in primary materials

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Near Infrared Reflectance Spectroscopy (NIRS), coupled with fiber optic probes, has been shown to be a quick and reliable analytical tool for quality assurance and quality control in the pharmaceutical industry, both for verifications of raw materials and quantification of the active ingredients in final products. In this paper, a typical pharmaceutical product, hydrocortisone sodium succinate, is used as an example for the application of NIR spectroscopy for quality control. In order to develop an NIRS method with higher precision and accuracy than the official UV/VIS spectroscopic method (BP '99), 19 samples, taken from one year's production and several prepared in the laboratory, having a hydrocortisone sodium succinate concentration in the range from 89.05 % to 95.83 %, were analysed by NIR and UV/VIS spectroscopy. Three frequency ranges: 5939.73–5627.32 cm^{-1}; 4863.64 – 4574.36 cm^{-1}; 4308.23–4200.24 cm^{-1}, with the best positive correlation between the changes in the spectral and concentration data, were chosen. The validity of the developed NIRS chemometric method for the determination of the hydrocortisone sodium succinate concentration, constructed by the partial least squares (PLS) regression technique, is discussed. A correlation coefficient of 0.9758 and a standard error of cross validation (RMSECV) of 1.06 % were found between the UV/VIS and the NIR spectroscopic results of the hydrocortisone sodium succinate concentration in the samples.

Keywords: FTNIRS, quantitative analysis, hydrocortisone.

INTRODUCTION

Basic theory of the Near Infrared Spectroscopy

Absorption bands relating to many chemical bonds, such as: C–H, N–H, O–H, S–H, C=O and C=C, are found in the NIR region, from 12800 to 4000 cm^{-1}. The NIR spectrum shows overtone and combination bands of these groups. Unfortunately, the absorption bands in the NIR region are broad and overlap, which means that conventional univariate calibration techniques, using only one wavelength per component for evaluations, cannot be applied in cases of overlapping bands. The development of more sophisticated statistical tools, like the most widely used partial least square (PLS) regression multivariate method for analysis, gave the possibility for the broad application of NIR spectroscopy to many analytical laboratories.

NIR spectroscopy offers a number of advantages for qualitative and quantitative analysis and process control applications, such as: no sample preparation, no waste, reduced costs, fast measurements and analysis, non-hygroscopic optical components, fiber optics for remote measurements, high analysis accuracy and ease of use.

Quantitative analysis

With the near-infrared reflectance technique, the sample to be analyzed is subjected to NIR radiation; the sample surface layers absorb part of the energy, while the rest is dispersed in all directions. The dispersed light gives information about the composition of the test sample and the resulting spectrum is used in qualitative and quantitative analysis.[1,2]

The best frequency intervals are in the region of the spectrum in which the reflected light intensity best correlates with the concentrations of the analyzed chemical species.

The goal for every quantitative analytical spectroscopic measurement technique, is to determine a certain system property – *Y* (%, concnetration value) from some measured system parameter – *X* (spectral data). The individual parameters *X* and *Y* are written in martix form. The spectral intensities, in the chosen frequency intervals, of the spectroscopic measurement are written point by point row-wise into the *X*-matrix. The spectral data for each measured sample correspond to one row in the matrix. The corresponding sample component concentration values are written in a similar way as the rows of the *Y*-matrix.

Such a quantitative analysis requires calibration and analysis (prediction). During the calibration, known samples are used to establish a certain relationship between *X* and *Y* parameters and calibration function *b*.

After the calibration, the analysis begins. During the analysis the system property *Y* is determined for new unknown samples by applying of the calibration model to the measured properties *X*:

$$Y = Xb$$

in which the calibration function *b* is given by:

$$b = (X^T X)^{-1} X^T Y$$

where *T* is used to denote the transponse of the associated matrix.

The most widely used method of calibration is Partial Least Squares (PLS)–Regression.[3–8]

EXPERIMENTAL

Apparatus

All the NIR spectra in this work were recorded from 12800 to 4000 cm^{-1} with a fiber optic probe connected to the FT-NIR spectrometer (Bruker Vector 22/N-F). Immersing the probe directly into the powder, four diffuse reflectance spectra of each powder sample were collected. A total of 16 scans, with the spectral resolution set at 8 cm^{-1}, were collected and averaged for both background and sample measurements. The background was taken with a *Spectralon* reference standard.

The UV/VIS spectra were measured with a CARY 1E UV-VIS spectrophotometer.

Data processing

The program OPUS/QUANT-2 with the OS/2 operating system was used for the quantitative analysis.

Preparation of samples

A set of samples belonging to one year's production batches was selected. To widen the spread of the concentration of the active ingredient, exact quantities of anhydrous sodium carbonate were added.[9] The concentration of the active ingredient, hydrocortisone sodium succinate as estimated by UV/VIS spectroscopy, ranged from 89.05 % to 95.83 %.

Determination of nominal value

The concentration of hydrocortisone sodium succinate was determined using the following method according to the *British Pharmacopoeia*, edition 1999 (BP '99): The analyzed powder was dissolved in sufficient water to produce a solution containing the equivalent of 0.001 % w/v of hydrocortisone. The absorbance at the absorption maximum of 248 nm of the resulting solution was measured. The content of hydrocortisone was calculated by taking an absorptivity of 449 as the value of *A* (1 %, 1 cm) at the 248 nm absorption maximum.

The reasons for low precision and accuracy of the results obtained using UV/VIS spectroscopic method (BP '99) for the determination of the content of hydrocortisone sodium succinate were also examined. There are several potential reasons for the low precision and accuracy of this UV/VIS spectroscopic method:

– The poor correlation between changes in the concentrations of hydrocortisone in the examined solutions and changes of the absorption intensity at 248 nm;
– The low stability of the prepared solutions, which can lead up to the variability of the results, if the absorptions of the prepared solutions were measured after different times of the preparation of each solution;
– The different preparation methods of the examined solutions, which can lead to different errors in measuring the appropriate amount of the to be examined powder and in the dilution from a high to the final concentration. As the concentrations of hydrocortisone in the final solution are very low (0.001 %), errors in the preparation of the examined solutions have a big influence on absorption intensity at 248 nm and hence, on the final results.

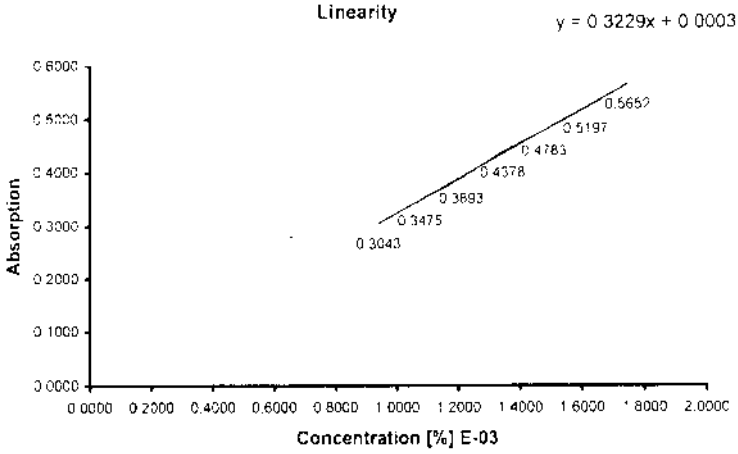

Fig. 1. Absorption *vs*. concentation of hydrocortisone sodium succinate using the UV/VIS spectroscopic method.

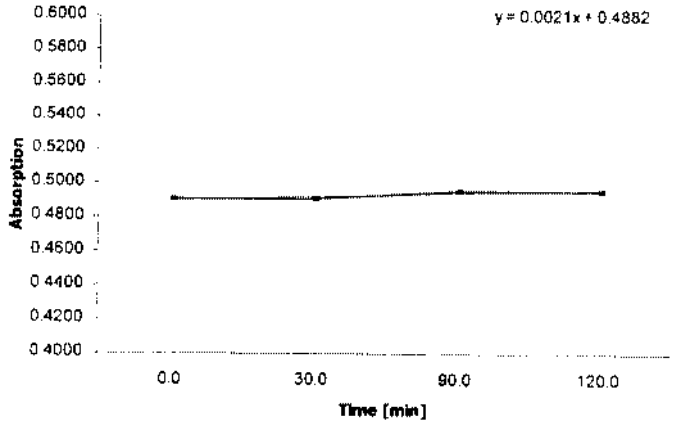

Fig. 2. Absoption *vs*. time of measuring of the hydrocortisone sodium succinate solution.

The examined UV/VIS spectroscopic method showed a good correlation between the changes in the concentrations of hydrocortisone in the examined solutions and the changes of absorption intensity at 248 nm with the high correlation factor of 0.9998. (Fig. 1).

An acceptable stability of the prepared solutions was also found. (Fig. 2). In order to compare the results obtained from two different preparation methods of the examined solutions, the Student *t*-test and correlation analysis were done.

Method 1: About 10 mg of the substance to be examined is dissolved in water to produce 1000 ml of solution. Method 1 avoids any error in diluting from a high to the final concentration, but a big error in the final results is introduced because of the very small amount of the to be examined powder that has to be weighed.

Method 2: About 27 mg of the to be examined substance is dissolved in water to produce 200 ml of solution. 10 ml of the prepared stock solution is diluted with water to produce 100 ml of final solution. The final solution is made in triplicate from the same stock solution and the absorbance at 248 nm of all three solutions is measured. The average results calculated.

The error introduced in measuring the appropriate amount of the to be examined powder on final result is smaller because the amount is almost three times higher than in Method 1. The influence of the error in diluting from a high to the final concentration on the final result is very small. The procedure is repeated in triplicate and an average result calculated.

As the t – value ($t = 3.729$) calculated by the Student t-test (Table I) is higher than the critical t - value ($t = 2.447$ for $= N–1 = 6$, $P = 0.05$) it can be concluded that a statistically important difference between the results obtained with Method 1 and Method 2 exists.

Furthermore, the correlation analysis for the results obtained with Method 1 and Method 2 showed a poor correlation between these two groups of results, with the low correlation factor of 0.9227.

Because of existing difference between the results obtained with Method 1 and Method 2, it is important to make the choice of the optimal method for the preparation of the to be examined solution. According to the previously described procedures for the preparation of the test solutions, it can be concluded that Method 2 has a smaller influence on the errors due to the preparation of the solutions on the results of the analysis, than Method 1. Hence Method 2 was selected to be the optimal method for the preparation of 0.001 % hydrocortisone solution for the UV/VIS spectroscopic quantitative analysis of hydrocortisone sodium succinate powder for injection.

TABLE I. The values of the parameters included in the Student *t*-test

Parameters	d	$\bar{d}$	Sd_d	t value
Equation	$\%HSS_{method\ 1} - \%HSS_{method\ 2}$	$\frac{d}{N}$	$\sqrt{\frac{d\ \bar{d}^{\ 2}}{N\ \ 1}}$	$\frac{\bar{d}}{\frac{S_{d_d}}{\sqrt{N}}}$
Sample No.	Method 1 HSS/%	Method 2 HSS/%	d	d^2
1	95.850	96.317	–0.467	0.218
2	95.070	93.636	1.434	2.056
3	94.237	92.977	1.260	1.588
4	96.055	94.136	1.919	3.683
5	93.090	91.652	1.438	2.068
6	94.162	91.257	2.905	8.439
7	91.586	88.630	2.956	8.738
d	d^2	Average d	Sd_d	t value
11.445	26.789	1.635	1.160	3.729

N – number of samples

RESULTS AND DISCUSSION

The calibration spectra were recorded of 19 hydrocortisone sodium succinate samples with hydrocortisone sodium succinate concentrations within the range from 89.05 % to 95.83 %. Four spectra were recorded. To set up a PLS calibration model, a total of 76 spectra were collected and the true concentration values of the hydrocortisone sodium succinate, obtained using the UV/VIS spectroscopic method (BP '99), were entered in the concentration data.

Three frequency ranges (5939.73–5627.32 cm^{-1}; 4863.64–4574.36 cm^{-1}; 4308.23 – 4200.24 cm^{-1}) with the best positive correlation between the changes in the spectral and concentration data were chosen (Fig. 3). The best positive correlation between the changes in the spectral and concentration data are in the frequency ranges with maximal absorption and the largest value for the coefficient of determination R^2. In the frequency range (5300–5000 cm^{-1}) a negative correlation between the changes in the spectral and concentration data exist because water molecules have a high absorptivity (1.6 $cm^2\ mol^{-1}$) in this region. This fact indicate that an increase in the concentration of water in the sample can yield to a decrease in the concentration of hydrocortisone sodium succinate in the sample and to a increased absorption in the frequency range from 5300 to 5000 cm^{-1}.

The Bruker QUANT-2 software package offers many options for data preprocessing, such as: no spectral data preprocessing, constant offset elimination, vector normalization, straight line subtraction, min-max normalization, multiplicative scatter correction, internal standard, first derivative and second derivative. The optimal method will depends on the system studied. Experience shows that in most cases a straight line subtraction followed by a vector normalization of the spectra of their first

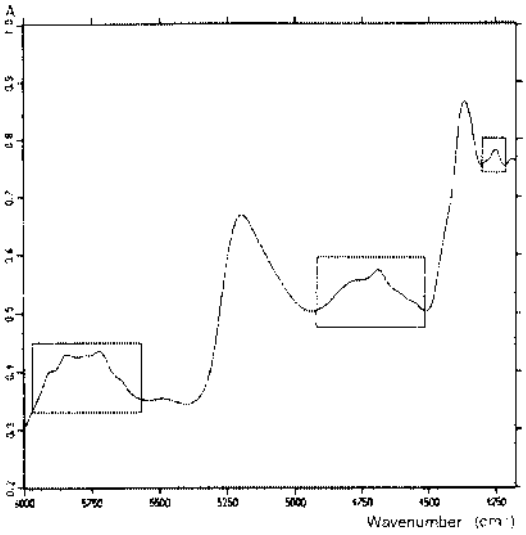

Fig. 3. FTNIR spectra of hydrocortisone sodium succinate. The marked spectral regions indicate the selected frequency ranges used for quantitative analysis.

derivatives will produce the best calibration models with the highest values for the coefficient of determination R^2.

For this study of hydrocortisone sodium succinate, the straight line subtraction (SLS) preprocessing method was found to give the best results for the PLS calibration model. In the PLS regression, the spectral and concentration data are first encoded in matrix form and then reduced to a few factors. The number of factors in the chemometric model is termed the "rank". In this chemometric model, automatically formed after forming the spectral and concentration data and after choosing the optimal frequency ranges and preprocessing method, the optimal rank is 10. With ten factors the maximum value for the coefficient of determination (R^2) (Fig. 4) and the minimum value for the root mean square error of cross-validation (RMSECV) (Fig. 5), were found.

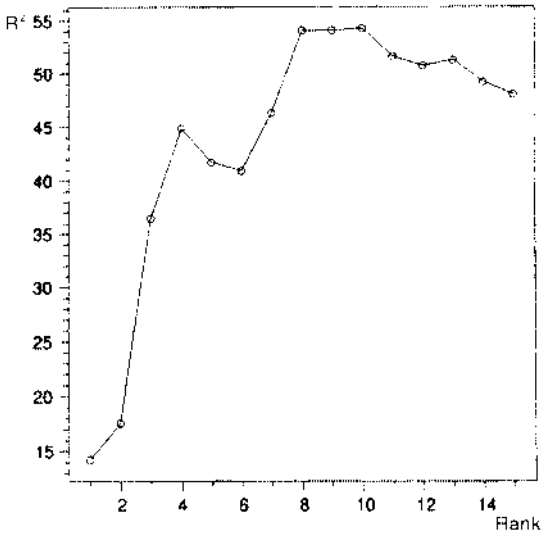

Fig. 4. R^2 *vs*. rank.

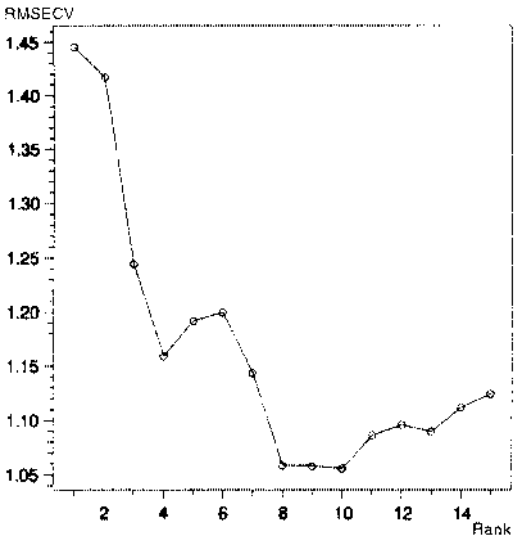

Fig. 5. RMSECV *vs*. rank.

The formed chemometric model was validated with a certain number of samples with known active ingredient concentration, using the chemometric model for the prediction of the concentration values. Comparison of the resulting predicted concentration values with the actual ones was used to determine the model parameters that are essential for checking the quality of the chemometric model.[10]

The calibration process included the construction of the final version of the chemometric model. The final version of the chemometric model is constructed only after all outliers have been removed from the calibration sample set and all system parameters have been determined. During the calibration process, the scores- and loading-vectors were calculated, as well as the calibration function *b*. The resulting values, stored in databases, are available for the analysis of new samples.

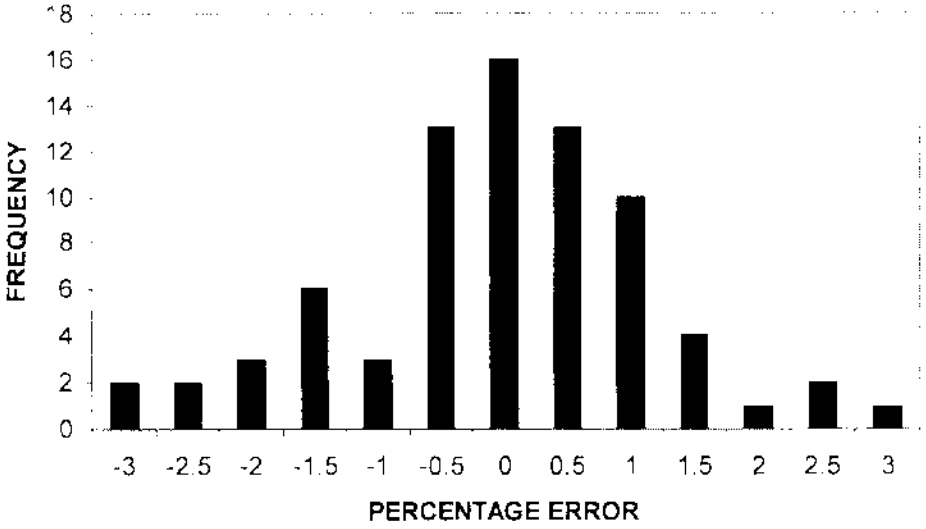

Fig. 6. Distribution of the percent error in the NIRS analysis of hydrocortisone sodium succinate.

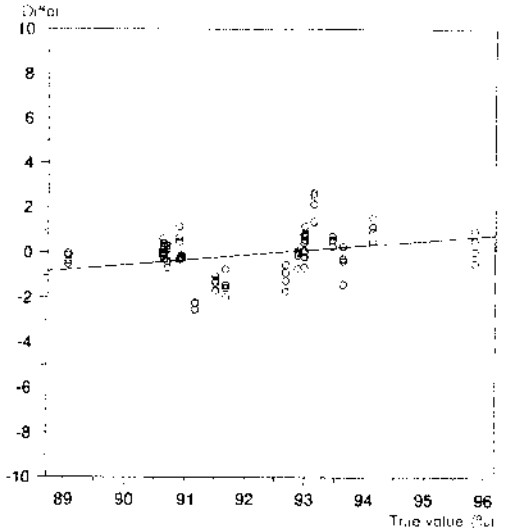

Fig. 7. Difference *vs*. true values of the hydrocortisone sodium succinate content.

The results obtained for the quantitative analysis of hydrocortisone sodium succinate show that the percentage errors have a good random distribution (Fig. 6), and that all fall well within the 3 % range normally considered acceptable for primary materials. Additionaly, the standard deviation calculated from the UV//VIS spectroscopic results (0.30 %) is higher than the standard deviation from the NIRS results (0.25 %). This fact indicates that the precision of the NIRS method is higher than that of the UV/VIS spectroscopic method.

Also, the validation and callibration results obtained from the formed chemometric model (Table II) show that the chemometric model is valid; that is, a high coefficient of determination – R^2 (higher than 90 %), a low value of errors – RMSEE, RMSECV and a high value of the correlation coefficient – R.

TABLE II. System parameters obtained with the NIR spectroscopic chemometric model for hydrocortisone sodium succinate

Parameters[10]	Equation	Obtained values
FValue	$\frac{(N \quad 1)\ (Spec\ Res_i)^2}{\sum_{j\ 1} (Spec\ Res_j)^2}$	1.0
FProb	$\frac{\int_0^{FValue} f(FValue)\,d(FValue)}{\int_0 f(FValue)\,d(FValue)}$	0.5
Sum of square errors (*SSE*)	$\sum_{i\ 1}^{N} (Res_i)^2$	8.87

TABLE II. (Continued)

Parameters[10]	Equation	Obtained values
Coefficient of determination (R^2)	$1 \quad \frac{SSE}{\sum_{i\ 1}^{N} (Y_i \quad Y_m)^2} \quad 100$	95.21
Root mean square error of estimation (*RMSEE*)	$\sqrt{\frac{SSE}{N \quad R \quad 1}}$	0.369
Root mean square error of cross-validation (*RMSECV*)	$\sqrt{\frac{1}{N} \sum_{i\ 1}^{N} (Y_i^{\text{meas}} \quad Y_i^{\text{pred}})^2}$	1.06
Correlation factor (R)	$\frac{\sum_{i\ 1}^{N} (Y_i^{\text{meas}} \quad Y_i^{\text{pred}}) \quad N \quad \overline{Y_i^{\text{meas}}} \quad \overline{Y_i^{\text{pred}}}}{\sqrt{\sum_{i\ 1}^{N} Y_i^{\text{meas}^2} \quad N \quad \overline{Y_i^{\text{meas}}}^2 \quad \sum_{i\ 1} Y_i^{\text{pred}^2} \quad N \quad \overline{Y_i^{\text{pred}}}^2}}$	0.9758

N – number of sample; i – sample index; $SpecRes_i$ – spectral residual of sample i; Res_i – residual of concentration data of sample i; Y_i – concentration value of sample i; Y_m – mean concentration value; X_i – spectral data of sample i; R – rank Y_i^{meas} - measured (true) concentration value of sample i; Y_i^{pred} – predicted concentration value of sample i

In addition, it can be concluded that a decrease of an increase in the difference between the true (UV/VIS spectroscopic) and predicted (NIRS) concentration value with changes in the true value of the hydrocortisone sodium succinate concentration (Fig. 7) does not really exist.

CONCLUSION

In modern medical therapy hydrocortisone plays a very important role and has a wide range of application. As a consequence of this, it is very important to provide fastest and more accurate and precise quantitative method for the analysis of hydrocortisone in samples.

Due to the variability of the result in the UV/VIS determination of hydrocortisone sodium succinate, the use of NIRS analysis was shown to be a more appropriate quantitative spectroscopic tool for such determinations. This characteristic, together with the fact that the raw material is analysed directly as such and the rapidly (20 s) obtained instrumental responses, confirm the appropriateness of the FTNIR spectroscopy for use in many pharmaceutical quality control laboratories.

И З В О Д

ПРИМЕНА БЛИСКЕ ИНФРАЦРВЕНЕ РЕФЛЕКСИОНЕ СПЕКТРОСКОПИЈЕ ЗА ОДРЕЂИВАЊЕ ХИДРОКОРТИЗОН НАТРИЈУМ СУКЦИНАТА

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Блиска инфрацрвена спектроскопија, метода дифузне рефлексије (NIRS), омогућава веома брзу и квалитетну квантитативну и квалитативну анализу сировина, полупроизвода и готових производа у фармацеутској индустрији. У овом раду је обрађена могућност примене блиске инфрацрвене спектроскопије у контроли квалитета фармацеутске сировине - хидрокортизон натријум сукцината за инјекције. У циљу развијања NIRS метода са већом прецизношћу и тачношћу од, до сада коришћеног, UV/VIS спектроскопског метода (BP '99), извршена је UV/VIS и NIR спектроскопска квантитативна анализа на 19 узорака, узетих из последње године производње и неколико припремљених у лабораторији, са интервалом концентрација хидрокортизон натријум сукцината од 89,05 % до 95,83 %. Изабрани су фреквенциони интервали: 5939,73–5627,32 cm^{-1}; 4863,64–4574,36 cm^{-1}; 4308,23–4200,24 cm^{-1}, са најбољом позитивном корелацијом између промена у концентрацији активне компоненте и промена у вредности апсорбанције узорака. Извршен је и процес валидације новоформираног NIRS квантитативног модела, који је конструисан регресионим методом најмањег квадрата. На основу вредности концентрација хидрокортизон натријум сукцината у испитиваним узорцима, добијеним UV/VIS и NIR спектроскопским методом, израчунати су одговарајући параметри, између осталог коефицијент корелације од 0,9758 и средња грешка укрштене валидације (RMSECV) од 1,06 %, који указују на задовољавајући квалитет формираног квантитативног NIRS модела за одређивање садржаја хидрокортизон натријум сукцината.

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REFERENCES

1. P. Kubelka, F. Munk, *Z. Tech. Phys.* **12** (1931) 593
2. P. Kubelka, *J. Opp. Soc. Am.* **38** (1948) 448
3. D. M. Haaland, E. V. Thomas, *Anal. Chem.* **60** (1998) 1193
4. G. Schwedt, *Taschenatlas der Analytik,* Thieme Press, Stuttgart, 1st ed: p. 110 ff (1992)
5. P. Geladi, B. R. Kowalski, *Anal. Chim. Acta* **1** (1986) 185
6. K. R. Beebe, B. Kowalski, *Anal. Chem.* **59** (1987) 1007A
7. H. Martens, T. Naes, *Multivariate Calibration,* Wiley, New York, 1989, Chapater 3.5
8. S. D. Brown, *Appl. Spectrosc.* **49** (1995) 14A
9. S. Lonardi, R. Viviani, L. Mosconi, M. Bernuzzi, P. Corty, E. Dreassi, C. Murratzy, G. Corbini, *Pharmac. & Biomed. Anal.* **7** (1989) 303
10. *Multivariate Calibration,* Bruker Optics, 2nd revised ed: (1999) p. 3.