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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 51–55
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Multivariate analysis of nystatin and metronidazole in a semi-solid matrix by means of diffuse reflectance NIR spectroscopy and PLS regression

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Received 24 March 2005; received in revised form 18 May 2005; accepted 20 May 2005
Available online 15 August 2005

Abstract

A multivariate method of analysis of nystatin and metronidazole in a semi-solid matrix, based on diffuse reflectance NIR measurements and partial least squares regression, is reported. The product, a vaginal cream used in the antifungal and antibacterial treatment, is usually, quantitatively analyzed through microbiological tests (nystatin) and HPLC technique (metronidazole), according to pharmacopeial procedures. However, near infrared spectroscopy has demonstrated to be a valuable tool for content determination, given the rapidity and scope of the method. In the present study, it was successfully applied in the prediction of nystatin (even in low concentrations, ca. 0.3–0.4%, w/w, which is around 100,000 IU/5g) and metronidazole contents, as demonstrated by some figures of merit, namely linearity, precision (mean and repeatability) and accuracy.

Keywords: NIR diffuse reflectance; PLS regression; Content determination; Nystatin; Metronidazole

1. Introduction

Nystatin and metronidazole (Fig. 1) are used as antifungal and antibacterial agents, widely applied in the treatment of vaginal infections. A common practice in a pharmaceutical industry is the use of microbiological and HPLC-based techniques to assess the content of drugs in a medicine, and these are the US Pharmacopeial methods applied to content determination of nystatin (microbiological) and metronidazole (HPLC). The ranges of acceptance using these methods of analysis are 90–130% for nystatin and 95–105% for metronidazole, but they are usually very time-consuming. Thus, a method capable to rapidly obtain the content of nystatin and metronidazole, in a reliably and simultaneously manner, is of great interest to pharmaceutical industries.

The use of near infrared (NIR) spectroscopy methods to assess quantitative information of drugs in medicines has increased in recent years [1–5]. Other spectroscopic techniques, such as UV and fluorescence, together with multivariate treatment, have also been applied in quantitative studies, requiring solutions preparation to proceed with measurements [6–9]. NIR spectra, when treated using chemometric methods of analysis, such as partial least squares (PLS) regression, may provide identification and quantification of compounds in a second or higher order mixture, with little or no sample preparation. This may be achieved by developing a calibration model and correlating the instrumental responses with the property of interest. Multivariate calibration methods allow simultaneous determination, non-resolution analyses and analysis even in the presence of interfering agents, since they are present in the calibration samples [5]. In order to build a calibration model, a data matrix **X**, formed by the diffuse reflectance NIR spectra of nystatin and metronidazole-based products, can be decomposed into latent variables by

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doi:10.1016/j.jpba.2005.05.025

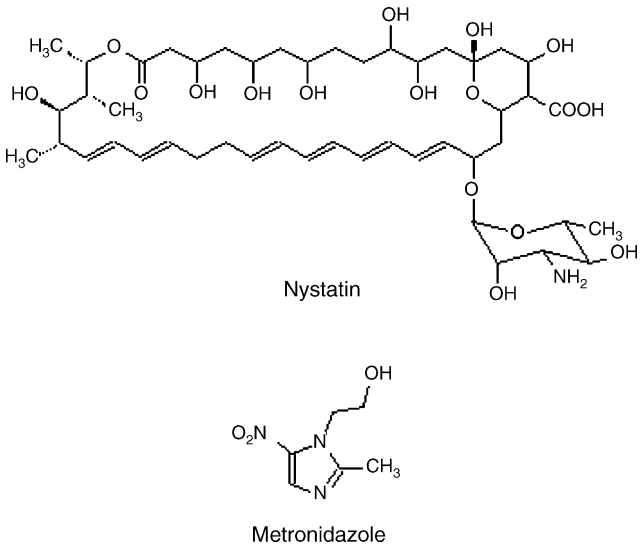

Fig. 1. Structures of nystatin and metronidazole.

PLS and the new regressors (the score vectors that replaced the original variables) correlated with the Y-block (dependent variables).

Then, the aim of this work was to built a multivariate calibration model for simultaneous content determination of nystatin and metronidazole in a semi-solid matrix, by using diffuse reflectance NIR spectroscopy, providing a faster procedure of analysis than the usual USP methods and keeping from same to better quantification capability.

2. Experimental

Calibration and test samples were prepared in order to meet from 80 to 120% of active concentration (0.35%, w/w of nystatin and 10.0%, w/w of metronidazole correspond to 100%, of active concentration). Nystatin and metronidazole were dispersed in propylene glycol and added to a mixture of cetostearyl alcohol, ethoxylated cetostearyl alcohol, decyl oleate, propylparaben, methylparaben, EDTA, simethicone, glycerin and water.

Spectra were acquired using an Antaris NIR analyzer (ThermoNicolet), through diffuse reflectance from the bottom of vials containing the nystatin/metronidazole-based product, 32 scans and resolution of 8 cm^{-1}. Each of nine calibration samples and five test samples were analyzed in triplicate. Spectral treatment was performed using the TQ Analyst Version 6 software [10]. The calibration was carried out using the mean-centered spectra, after multi-scattering correction and first derivative transforms, over the region of 4000–10,000 cm^{-1}.

3. Results and discussion

Diffuse reflectance NIR spectra were obtained for nine calibration samples and five test samples (as a minimum recommended by regulatory agencies). The distribution of the calibration and test sets were arranged as follows (in percent of nystatin:metronidazole concentration):

Calibration samples: 80:80, 90:90, 100:100, 110:110, 120:120, 80:120, 90:110, 110:90 and 120:80;
Test samples: 105:85, 95:95, 85:105, 105:105 and 95:115.

Spectra from calibration samples were correlated with the dependent variables, the reference values, through PLS regression. Here, the reference values are the analytically weighted masses of nystatin and metronidazole used when manufacturing the studied batches (from ca. 80 to 120% of active concentration). External validation spectra were used to test the calibration model. Some figures of merit were taken into account in the present study in order to evaluate the prediction ability of the model, according to their respective definitions:

Linearity: According to the ICH [11] definition, linearity is the ability of a model (within a given range) to obtain test results that are directly proportional to the concentration of the analyte in a sample. This may be approximately estimated by correlating the predicted values versus the reference values, calculating the correlation coefficient, the *y*-intercept and the slope of the regression line [12].
Precision: It represents the degree of scatter between a series of measurements for the same sample under prescribed conditions. It is usually expressed as a standard deviation or relative standard deviation of a series of measurements [11,13]. The precision levels evaluated in this work were repeatability, obtained by the standard deviation of six measurements for a single sample made on the same day, and mean precision, that should be determined as the mean of the standard deviation of a minimum of six measurements on a minimum of three samples, in agreement with an appropriate work by Taverniers et al. [14].
Accuracy: This parameter reports the closeness of agreement between the reference value and the value found by the calibration model. In chemometrics, this is generally expressed as the root mean square error of the prediction (RMSEP) sample, that is an approximation of the standard error of the prediction samples [15].

Spectral contributions from the used matrix strongly superimpose on the active bands, as may be easily seen in Fig. 2. Thus, an analysis of loadings was performed through using the chemometric Pirouette 3.11 software [16], in order to evaluate the weights of each original variable (absorbances), corresponding to both nystatin and metronidazole, on the calibration model. Factor number one, which retains the most significant variance in the data, shows that higher loadings are given to the spectral region of lower wavenumbers (4000–6500 cm^{-1}), as illustrated in Fig. 3. However, selection of this region to run calibration did not improve the model quality and, then, the full spectral range was utilized for this purpose. Spectra were mean-centered, and multiplicative scatter correction followed by first deriva-

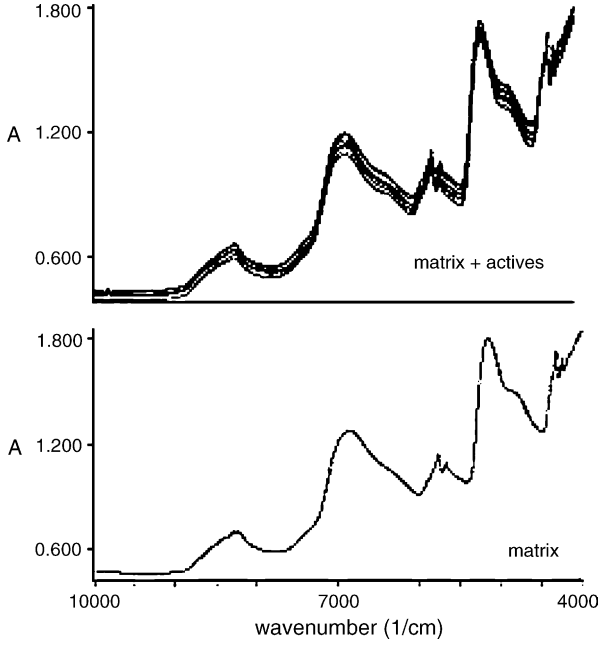

Fig. 2. Original spectra for calibration samples and for the semi-solid matrix.

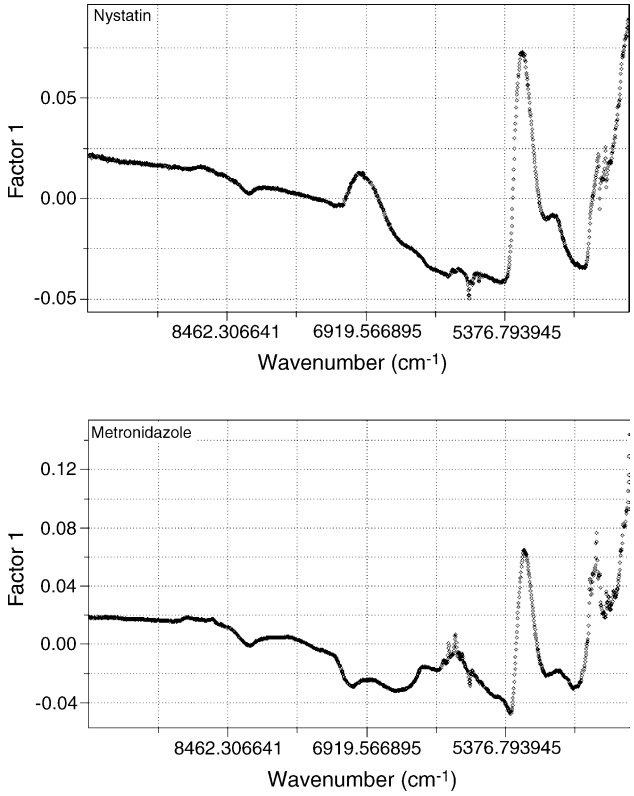

Fig. 3. Loading plots for nystatin and metronidazole.

tive transform was applied over them to provide a better modeling capability (Fig. 4).

Fig. 5 shows the plots of experimental versus calibrated values of percentage (w/w) of nystatin and metronidazole, as well as the residual plots demonstrating the absolute errors distribution of the calibration samples versus the reference values. These were obtained using the number of PLS components (five factors for nystatin calibration and four factors for metronidazole calibration) in which no significant changes could be observed on the cumulative percentage of explained variance, when increasing number of factors, and whose RMSECV (root mean square error of cross-validation) was sufficiently low. This calibration was applied to predict the content of nystatin and metronidazole in test samples and to evaluate the figure of merits shown in Table 1.

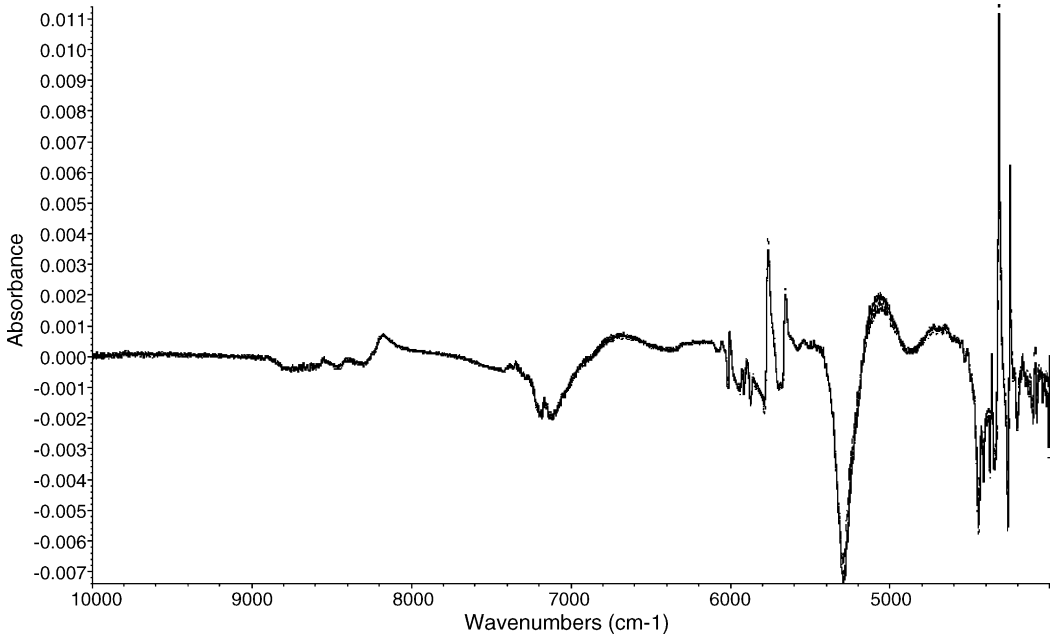

Fig. 4. First derivative and multi-scattering corrected spectra of calibration samples.

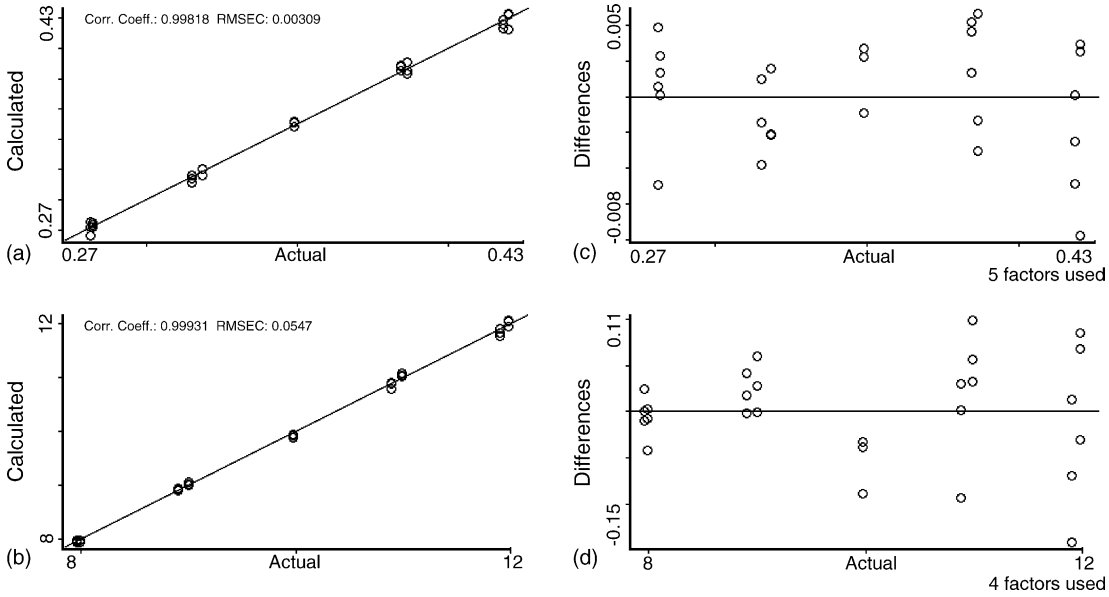

Fig. 5. Calibration and residual plots for (a) nystatin and (b) metronidazole.

In order to achieve the sensitivity of the method, experiments with placebo were carried out and the predictions showed that sensitivity is not a problem in our model, since the results were not within 80–120% of the nominal value of each active. 0.08%, w/w (23% of the nominal value) and 0.06% (0.6% of the nominal value) of nystatin and metronidazole, respectively, were found for the placebo sample. Although the nominal percentage of nystatin in placebo has been high, due to the narrow range in which the experiments were performed, it was not close to the 80–120% range.

The accuracy values showed an acceptable uncertainty level for the requirement of quality control for content determination, even for nystatin, where the concentration is ca. thirty times lower than for metronidazole. All external predictions demonstrated to be within the range of 95–105% of tolerance (Table 2), more restricted than the microbiological test for nystatin, where the tolerance is 90–130%. A comparison between RMSEC and RMSEP indicates that they are very close, suggesting that calibration and validation samples are exchangeable and have similar standard deviation. The values for slope, *y*-intercept and correlation coefficients were determined by a linear regression between the reference values against the predicted ones for the validation samples, and results near to the ideal were obtained (1, 0 and 1, respectively). Based on these data and the good results for the accuracy and precision, it may be concluded that the linearity of the model was accessed. Obviously, since metronidazole is more accessible to quantification due to its higher concentration in comparison with nystatin, its model is more parsimonious than that for nystatin (four factors used in calibration against five factors for nystatin) and the predictive ability of its model is also higher. Nevertheless, concentration of both actives might be satisfactorily determined, as demonstrated by the validation tests, and the method showed to be applicable, in the level of ±5%, even in contents of nystatin lower than 1%.

Table 1
Analytical figures of merit for the calibration model

Figures of merit	Nystatin	Metronidazole
Rank		
PLS components	5	4
Adjust		
R_{cal}	0.998	0.999
Slope	0.9953	0.9986
Intercept	0.0017	0.0143
Linearity		
R_{test}	0.967	0.996
Slope	0.8839	1.0083
Intercept	0.0499	−0.1430
Accuracy[a]		
RMSEC	0.0031	0.0547
RMSEP	0.0083	0.1150
Precision[a]		
Mean	0.0096	0.0590
Repeatability	0.0057	0.0452

[a] Results in percentage (w/w).

Table 2
Results of prediction for validation samples (%, w/w)

Sample no.	Actual		Predicted	
	Nystatin	Metronidazole	Nystatin	Metronidazole
1	0.366	8.42	0.363 ± 0.006	8.31 ± 0.10
2	0.335	9.50	0.346 ± 0.004	9.58 ± 0.12
3	0.294	10.35	0.309 ± 0.006	10.20 ± 0.11
4	0.367	10.43	0.384 ± 0.006	10.30 ± 0.08
5	0.323	11.32	0.337 ± 0.003	11.33 ± 0.06

4. Conclusions

The developed method of analysis of nystatin and metronidazole, based on diffuse reflectance NIR spectroscopy and PLS regression, compares well with the pharmacopeials, and presented the advantages of offer instantaneous and simultaneous quantitative results. In addition, it demonstrated to be more rigorous than the microbiological range of determination (90–130%), since the model for nystatin was able to predict contents for test samples within 95–105% of accuracy uncertainty.

Acknowledgement

EMS Sigma Pharma is gratefully acknowledged for the support of this research.

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