ANEXO VI

Near infrared transflectance spectroscopy. Determination of dexketoprofen in a hydrogel

M. Blanco y M. A. Romero

Enviado para su publicación

NEAR INFRARED TRANSFLECTANCE SPECTROSCOPY. Determination of dexketoprofen in a hydrogel

M. Blanco and M.A. Romero
Departament de Química, Unitat de Química Analítica, Facultat de Ciencies,
Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain

ABSTRACT

A new method for the determination of the active principle dexketoprofen in a hydrogel using near infrared spectrometry was developed. A transflectance module allows the gel spectrum to be recorded with no sample pretreatment. Also, the PLS calibration method used allows low contents of the active principle in such a highly absorbing matrix as water/ethanol to be determined. The proposed method was validated and found to be an effective choice for the intended purpose.

Keywords: Near infrared spectroscopy, transflectance, dexketoprofen, hydrogel.

1.Introduction

The use of near infrared spectroscopy (NIR) for the analysis of pharmaceuticals has grown steadily over the last few years in terms of both the number of applications and their diversity. The ability to perform direct measurements with no sample pretreatment and the expeditiousness with which results can be obtained have turned NIRS into a highly useful analytical tool for pharmaceutical quality control in a variety of situations.[1] Reported applications include the construction of libraries for the identification of raw materials, intermediate products and finished products;[2,3] the development of methods for the quantitation of active principles and/or excipients,[4,5]and the on-line monitoring of blending of pharmaceutical ingredients.[6]

A lot of methods for determination of active principles and/or excipients in commercially available pharmaceutical preparations have been developed most of which are applied to solid products. Dedicated diffuse reflectance NIR modules for different

sample forms (tablets, powders, granulates) allow sample spectra to be obtained directly from the solid with no pretreatment. On the other hand, NIR transmission measurements have traditionally been used for the analysis of liquid samples such as syrups and oral preparations; lately, however, they have also been employed with intact tablets.[7]

One alternative method for recording NIR spectra is the transflectance mode, in which the incident light crosses the sample, is reflected off a piece of a material such as stainless steel, gold or PTFE located on the opposite side, and travels back through the sample before reaching the detector; the light path is roughly twice longer than the distance between the entry point and the reflector. Near infrared transflectance measurements have so far been used for the analysis of liquid or semi-solid samples. However, no application to pharmaceutical methods appears to have been reported to date.

The NIR spectrum for a gel can be recorded neither by transmission - filling and emptying of the cuvette are both slow and cumbersome - nor by reflectance - it is impossible to accurately establish the light path. On the other hand, transflectance measurements allow gel spectra to be easily and expeditiously obtained.

This paper demonstrates the suitability of NIR transflectance measurements for analytical quality control of transparent or semi-transparent semi-solid pharmaceuticals such as gels and pomades. The proposed method allows the determination of the active principle dexketoprofen in a water/alcohol matrix, which exhibits high absorption in the NIR region; also, the product has a low content in the analyte, which makes it difficult to construct an accurate calibration model. The model developed in this work was validated with a view to confirming its suitability for the determination of the active principle in the pharmaceutical preparation.

2. Experimental

2.1. Hardware and software

Spectra were recorded on a NIRSystems 6500 spectrophotometer from Foss NIRSystems (Raamsdonksveer, The Netherlands) that was equipped with a Rapid Content Analyzer (RCA) module, furnished with a gold reflector (optical spacing, 0.5 mm). The instrument was governed and data acquired using the Vision 2.22 software package, also from Foss NIRSystems. PLS models were constructed using Unscrambler 7.5, from CAMO (Trondheim, Norway).

2.2. Samples

The sample was a gel containing 12.5 mg/g of the active principle (dexketoprofen) in addition to water and ethanol as excipients

(*ca.* 95%), a gelling agent and a flavour. An overall 14 samples from different production batches were used. All were supplied by Laboratorios Menarini (Badalona, Spain).

In order to expand the concentration range spanned by the production samples, 16 laboratory samples were prepared by overdosing or underdosing production samples with the active principle or water/ethanol mixtures in different proportions, respectively.

2.3. Recording of NIR spectra

An appropriate amount of gel was placed in a flat-bottom quartz or glass cuvette and the reflector positioned in its bottom (Fig. 1). A blank signal was previously obtained in the absence of gel. Each recorded spectrum was the average of 32 scans over the wavelength range 1100–2500 nm performed at 2 nm intervals.

2.4.UV reference procedure

The dexketoprofen (DKP) content in the production and doped samples was determined by dissolving *ca.* 400 mg of sample in about 35 ml of 1:1 H_2O/MeOH in an ultrasonic bath for 10 min. The solution thus obtained was made to 50 ml with the previous mixture, and a 5 ml aliquot was diluted to 50 ml and used to record the UV spectrum. The content in the active principle was determined by using the spectrum for pure DKP in the same solvent as standard.

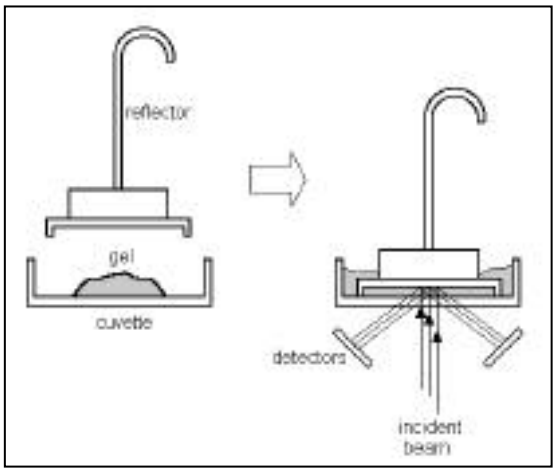

Figure 1. Schematic diagram of the transflectance mode using a gold plate reflector.

3. Results and Discussion

The calibration process required special care, and so did the preparation of the laboratory samples - exposure to the air must be reduced as far as possible to avoid losses of volatiles, which might affect the concentration of the active principle.

Samples were split into a calibration set and a prediction set. The calibration set consisted of 15 samples, *viz.* 10 laboratory samples spanning the concentration range from 9 to 15 mg/g (approximately ± 30% or the nominal content in the active principle) and 5 production samples. The prediction set also comprised 15 samples (9 production samples and 6 laboratory samples). The PLS1 algorithm was used to construct all models,

which were evaluated in terms of the mean square error of prediction (RMSEP).

The gel studied contained a nominal DKP concentration of 12.5 mg/g (1.25%) in a strongly absorbing matrix (60:35 water/ ethanol). Figure 2 shows the spectrum for a production sample, the active principle and a placebo containing all ingredients in the gel except the active principle. The spectrum for the placebo, which was virtually identical with that for the gel, exhibited two very strong bands in the 1300–1600 and 1850–2000 nm regions, both due to water, in addition to a series of weak bands in the 1650–1850 and 2200–2350 nm regions essentially due to ethanol. The low concentration of the active principle and the high absorption of the matrix concealed the contribution of dexketoprofen. Consequently, calibration models were constructed by using the wavelength ranges of maximum DKP absorption (1600–1800 and 2100–2500 nm) while avoiding those of strong matrix absorption.

Table 1 shows the result provided by the models tested. Those including the 2200–2500 nm range exhibited more complexity and a lower predictive ability, possibly as a result of the still high absorption by ethanol and water. The best model was based on second-derivative spectra over the 1600–1800 nm range; it provided results with a relative error of only 1.6% with respect to the reference values

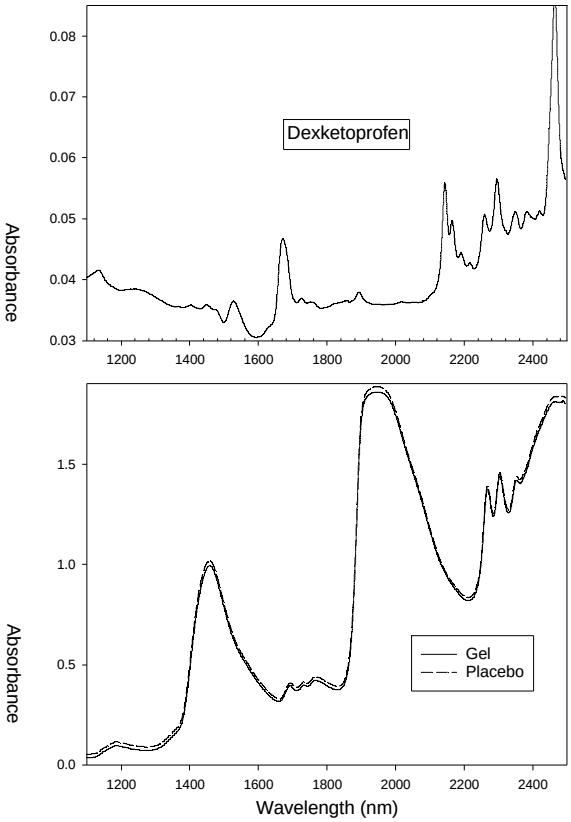

Figure 2. Spectra of Dexketoprofen and a production sample compared with a placebo of the formulation.

Table 1. Relative mean square error of prediction for Calibration and Prediction Sets

Spectra Pretreatment	Wavelength range (nm)	PLS factors	RMSEP(C)	RMSEP(P)
1st Derivative	1600-1800, 2200-2500	5	0.17	1.14
	1600-1800	5	0.11	0.25
	2200-2500	5	0.18	1.20
2nd Derivative	1600-1800, 2200-2500	5	0.05	1.85
	1600-1800	3	0.08	0.25
	2200-2500	5	0.06	1.86

The low sensitivity of NIR spectroscopy, which can hardly quantify analyte contents below 1%, together with the apparent absence of DKP absorption bands in the gel spectrum might question the goodness of the results provided by the proposed model. For this reason, a new calibration model (Extended model) was constructed from the same training set, which was expanded with a gel placebo containing no active principle. The results and predictive ability thus obtained essentially coincided with those of the original model. Table 2 shows the figures of merit for the plot of NIR *vs* reference values for the calibration samples in the original model and the placebo-extended model. In order to confirm the goodness of the latter, six laboratory samples with an active principle content of 1, 2, 4, 6, 7 and 8 mg/g - all below those used to construct the original model - were prepared. Application of the extended model yielded good linear fitting.

The fact that the new model accurately predicted concentrations 10 time lower than the nominal content testifies to the goodness of the original model. In order to confirm its reliability, the calibration model was validated.

3.1.Validation

Following the ICH guidelines,[8] the proposed method was validated by determining its selectivity, linearity, repeatability, intermediate precision, accuracy and robustness.

Table 2. Parameter of the curves: NIR value vs Reference value for original calibration set and for calibration set + placebo (extended model).

	Original Model	Extended Model
PLS Factors	3	3
Slope	0.997	0.998
Intercept	0.033	0.024
Correlation	0.998	0.999
RMSEP (Prediction Set)	0.25	0.24

3.1.1. Selectivity

The selectivity of the proposed method was determined by constructing a library with the spectra for the gel, the major constituents of the pharmaceutical preparation and the placebo - which included all ingredients except the active principle. The

discriminant criterion used was the residual variance for second-derivative spectra over the 1100–2500 nm range, using a threshold of 0.84. Table 3 shows the results obtained in the identification of all the components present in the library as belonging to the class defined by the gel. Gel samples were accurately identified (*i.e.* the identification value was always below the threshold); all others were above the threshold. A water/ethanol mixture in the same proportion as in the pharmaceutical (Water–ethanol 1) and two others with slightly different proportions (Water–ethanol 2 and 3) were clearly discriminated from the pharmaceutical.

Table 3. Identification of gel samples and major components, selected to be identified as the gel class. Threshold 0.84 (Positive Identifications: Id.Results<0.84).

SAMPLE	ID. RESULT
Gel	0.641
Water	0.999
Ethanol	1.000
Water-Ethanol 1	0.998
Water-Ethanol 2	0.999
Water-Ethanol 3	1.000
Placebo	0.960

3.1.2. Linearity

This parameter was assessed by plotting the NIR values against those provided by the reference method for 7 laboratory samples spanning the concentration range used for calibration. The graph was a straight line of slope and intercept not significantly different from 1 and 0 (Table 4), which confirmed the linearity of the PLS model throughout the concentration range studied.

3.1.3.Repeatability

The repeatability of the proposed method was evaluated by having the same analyst perform 6 determinations on the same production sample. Table 4 shows the results obtained. The coefficient of variation (CV) was 0.7%.

3.1.4. Intermediate precision

This was assessed in terms of two factors potentially affecting analyses, namely: day and analyst. To this end, the same production sample was analysed by two different operators over a three-day period (Table 4). The analysis of variance of the results revealed that neither effect was significant. The overall coefficient of variation was 1.1%.

3.1.5. Accuracy

The accuracy of the NIR method was estimated by using it in 9 determinations of samples from different production batches, the results being compared with those provided by the reference method. A *t*-test revealed the absence of significant differences between them (Table 4).

Table 4. Validation of the quantitative model for the determination of dexketoprofen in the hydrogel.

Parameter	Procedure	Results
Linearity	NIR value= a + b · Ref. value	n = 7 Range= 9-16 mg/g Curve parameters: Slope (b)= 0.97 ± 0.05 Intercept (a) = 0.11 ± 0.60 r = 0.988
Accuracy	t-Test of Differences of NIR values vs Reference Values in production samples	n = 9 Avg. Diference=0.12 Std Dev..= 0.19 t experimental = 1.89 t tabulated = 2.31
Repeatability	6 determinations of the same sample by the same operator	Mean = 12.21 Std. dev = 0.08 C.V. = 0.7 %
Intermediate Precision	1 sample analysed by two operators on three consecutive days	Mean = 12.32 Std. dev = 0.14 C.V. = 1.1 %
Robustness	Production Samples analysed over a period of a year.Comparison: NIR vs Reference Value	Relative error = 1.6 %

3.1.6. Robustness

The robustness of the proposed method was evaluated by analysing production samples over a one-year period and comparing the results with the reference values. As can be seen from Table 4, the proposed method provided consistent results over time, which testifies to its robustness.

4. Conclusions

The proposed method allows the rapid, direct NIRS determination of dexketoprofen in a hydrogel with no sample preparation or need for a solvent. Near infrared transflectance measurements provide results that are accurate enough to determine an active principle at low concentrations, with a relative error of only 1.6% for prediction samples.

The method was validated and found to be an effective alternative to the established UV spectrophotometric method for this purpose.

Acknowledgements

The authors are grateful to Spain's DGICyT for funding this research within the framework of Project BQU2000-0234. M.A. Romero also acknowledges additional funding from Spain's Ministry of Education and Culture in the form of a research training grant. Finally, the authors wish to thank Laboratorios Menarini (Badalona, Spain) for kindly supplying the pharmaceutical samples.

References

[1] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch and C. de la Pezuela, *Analyst*, **123**, 135R-150R (1998).

[2] C. I. Geräusser and K. A. Kovar, *Appl. Spectrosc.*, **51**, 1504-1510 (1997).

[3] P. K. Aldridge, R. F. Mushinsky, M. M. Andino and C. Evans, *Appl. Spectrosc.*, **48**, 1272-1276 (1994).

[4] D. Trafford, R. D. Jee, A. C. Moffat and P. Graham, *Analyst*, **124**, 163-167 (1999).

[5] M. Blanco, J. Coello, A. Eustaquio, H. Iturriaga and S. Maspoch, Anal.Chim. Acta **392**, 237-246 (1999).

[6] P. A. Hayley, P. Doherty, P. Tapsell, T. Olivier and P. K. Aldridge, *J. Pharm. Biomed. Anal.*, **14**, 551-559 (1996).

[7] P. Corti, G. Ceramelli, E. Dreassi and S. Mattii, *Analyst*, **124**, 755-758 (1999).

[8] *ICH Q2B: Validation of Analytical procedures: Methodology*, Consensus Guideline, International Conference on Harmonisation (ICH) (1998).