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Short communication

Direct determination of niflumic acid in a pharmaceutical gel by ATR/FTIR spectroscopy and PLS calibration

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Abstract

A simple, rapid and convenient analytical method without sample handling procedure is proposed for the determination of niflumic acid in a pharmaceutical gel with attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR). A partial least square (PLS) calibration model for the prediction of niflumic acid contents was developed using 81 and 27 spectra of standard gels as training and validation sets, respectively. The used spectral range of niflumic acid for the establishment of this model was 2300–1100 cm^{-1}. All spectra were obtained in the transmittance mode, then normalized and first derivative transformed. The model yielded a regression coefficient R^2 equal to 1 for the training set and a root mean square error of prediction (RMSEP) equal to 0.2 for the validation set. The percentage recoveries of the method for the analysis of NIFLUGEL® ranged from 96.60 to 101.02%.

Keywords: Niflumic acid; FTIR; Attenuated total reflectance; Partial least squares regression; Pharmaceuticals

1. Introduction

Niflumic acid (i.e. 2-[3-(trifluoromethyl) amino] nicotinic acid) (NFA) is the active principle in pharmaceutical formulation of NIFLUGEL®. This non-steroidal anti-inflammatory drug provides potent analgesic and anti-pyretic properties and is widely prescribed for treatment of rheumatic diseases [1]. Diverse pharmaceutical forms such as capsules, gels, ointments and suppositories are available to allow peroral or transcutaneous applications.

Various techniques such as sensitised luminescence [2], capillary isotachophoretic [3], HPLC [4–7] and gas–liquid chromatography methods [8] have already been used for quantitatively analysing NFA. However, HPLC and luminescence methods were devoted to quantification in biological samples (plasma, urine), and the accuracy and precision were thus unsuitable for quality control requirement. They appear relevant but somewhat expensive and time consuming methods. Sample preparation is often required prior to analysis. So, there is a need for a low-cost, simple and rapid method for NFA determination.

Fourier transform infrared spectroscopy analysis provides rapidly very interesting qualitative and quantitative informations. The attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR), originally developed by Fahrenfort [9], was suggested as a sensible technique to analyse pharmaceutical formulations such as solids, semi-solids and liquids [10,11]. However, very limited applications have been reported for quantification of drugs [12–16] although ATR is a method, which simplifies sample handling, avoids the use of organic solvents and thus saves time and chemical reagents. The main drawback of spectral data is the overlapped information contained in the signal for chemical mixtures. Furthermore, the relationship between the measured signal and the property of interest may result in non-linear correlations. In order to take advantage of spectroscopic technique, chemometric approaches such as partial least squares (PLS) regression have frequently been used in quantitative spectral analysis to obtain very selective information

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from unselective data [17,18]. PLS is particularly adapted to develop quantitative analysis of data from mixtures even if some components are unknown [19]. First, PLS needs a calibration step, where the relationship between the spectra and the concentrations of the components is deduced from a set of reference samples. Then, a prediction step consists in determination of the concentrations of the components from the spectra of the analysed samples from the results of the calibration [20–22].

The main objective of this work is to investigate the feasibility of ATR/FTIR spectroscopy associated with PLS regression used as the multivariate linear calibration method for predicting NFA amount in commercial gel formulation. The validation of the developed PLS model is performed. The method was evaluated for NFA content determination in commercial gels and the results were found between 95 and 105% of the labelled content as required in pharmaceutical industry [23].

2. Experimental

2.1. Apparatus

Spectra were obtained with a Mattson model Genesis II FTIR™ spectrometer controlled by Winfirst software from Mattson instruments Inc. (Win.Lab.Instruments, Bagnolet, France). This instrument is equipped with a Specaclamp Incompartment horizontal ATR from Benchmark series (P/N 11160) supplied with a top plate zinc selenide crystal that gives six internal reflections at a fixed angle of incidence of 45°.

2.2. Chemicals

NIFLUGEL® contains 25 mg/g (w/w) of NFA in a mixture of di-isopropanolamine, synthalen K, ethanol 96% and water. The pharmaceutical speciality of NIFLUGEL® and synthalen K were kindly donated by Bristol Meyers Squibb (Saint-Nazaire, France). Di-isopropanolamine and NFA were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France) and ethyl alcohol 96% from Carlo Erba (Val de Reuil, France). Distilled water was used to prepare reconstituted forms.

2.3. Sample preparation

In a tight closed container, synthalen K was introduced slowly in a mixture of distilled water and ethyl alcohol. The resulting solution was mixed 10–15 min with a mechanical stirring using a glass rod. A solution of di-isopropanolamine in distilled water was then added in the previous solution and mixed 5 min from top to bottom in order to obtain the neutral gel. Appropriate concentrations of NFA were progressively stirred by hand with neutral gel during 5 min. Gels were sealed with caps in glass flasks at room temperature until they were used. The final addition of NFA in the neutral gel was performed on the same day of the analysis.

2.4. Analytical method

Training and validation sets are composed of 27 and 9 standard gels, respectively. Each gel was prepared with neutral gel where NFA was added in order to obtain various gels at different concentrations ranging from 20 to 30 mg g^{-1} with increment steps at 1.25 mg g^{-1}. The set of samples with known compositions were measured as close as possible to the conditions where true measurements took place (i.e. from 80 to 120% of the labelled concentration of commercial product).

Each sample was spread on the ATR crystal without any prior treatment and was scanned. Between each measurement, the ATR crystal was carefully cleaned with distilled water and ethyl alcohol and then air dried. Single beam spectra of the samples were obtained after averaging 32 scans between 2300 and 1100 cm^{-1} with a resolution of 0.5 cm^{-1} and corrected against the background spectrum of the clean ATR crystal. All spectra were obtained in the transmittance mode. Then spectra were normalized by setting the minimum data value to zero. This difference between the minimum signal and zero was then substracted from all other data values. Next all spectra were first derivative transformed.

Data obtained from Winfisrt software were exported in ASCII format to Microsoft Excel. Calibration and quantification were done using the PLS model as provided in Simca P software (Version 9.0 of Umetrics, Umea, Sweden).

Variables were only centered. The optimum number of calibration factors was based on the regression coefficient R^2 for the training set and the RMSEP for the validation set. The predictability of the model was tested by application of this calibration model on NIFLUGEL®.

3. Results and discussion

Three series of gels containing nine different concentrations of NFA were individually prepared in order to check the homogeneity of gel preparation. Moreover, these 27 samples were then divided into three parts and analysed which constitute 81 objects. These training data are used to build PLS calibration. NFA concentrations constitute the Y observation matrix. The mean-centered normalized and first derivative transformed data of transmittance constitute the X variable matrix.

With attenuated total reflectance by FTIR spectroscopy, the penetration depth of the infrared beam in the sample is sufficiently large to insure a spectral reproducibility and thus a representative averaging of the gel. Furthermore, the gels were carefully prepared into a close environment in order to prevent the presence of air in the samples. First single beam spectra of the samples were obtained after Fourier transformation of interferogram measured between 4000 and

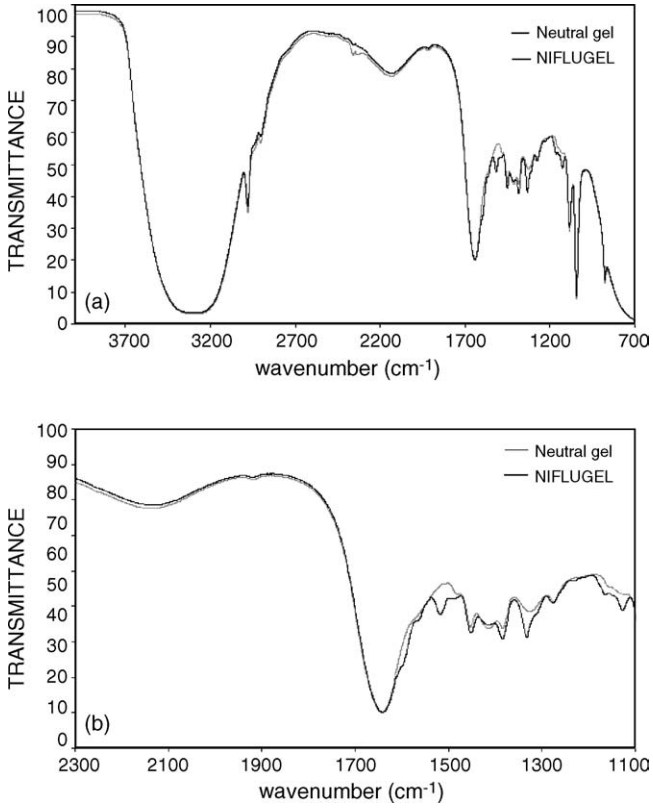

Fig. 1. Spectra in transmittance mode: (a) spectra obtained between 4000 and 700 cm^{-1} and (b) spectra obtained between 2300 and 1100 cm^{-1}.

700 cm^{-1} in the transmittance mode. Fig. 1a presents the ATR reflexion spectra of NIFLUGEL® and neutral gel over this spectral region. The neutral gel corresponds to a reconstituted gel without NFA, i.e. a gel containing only the excipients.

Although the gels were prepared in a close environment in order to prevent air content, the region between 4000 and 2300 cm^{-1} was eliminated where CO_2 contents is detectable at ±2350 cm^{-1}. The region between 1100 and 700 cm^{-1} was also eliminated prior to the calculations since the region 2300–1100 cm^{-1} shows systematic change due to the concentration of NFA (Fig. 1b). Moreover, reducing the spectral range decreases the number of variables, thus reducing data storage.

The spectra were normalized and then first derivative transformed, which eliminates the background noise. These transformations in combination could remove spectral variation not due to NFA concentration and allowed constructing the calibration model. The final variable number was 2401 since the signal was collected each 0.5 cm^{-1} over the range 2300–1100 cm^{-1}. Fig. 2 shows the variation of NFA amount within the calibration set along the selected spectral region.

Using the calibration model, the amount of NFA in a validation set composed of 27 samples (i.e. 9 gels with different concentrations of NFA divided into 3 parts and then analysed) was estimated.

To determine the number of significant factors of PLS model, we have determined for each component the regression coefficient R^2 for the training set and the RMSEP for the validation set. As shown in Fig. 3, the number of significant factors is equal to 14 and corresponds to the higher value of R^2 and to the minimum value of RMSEP.

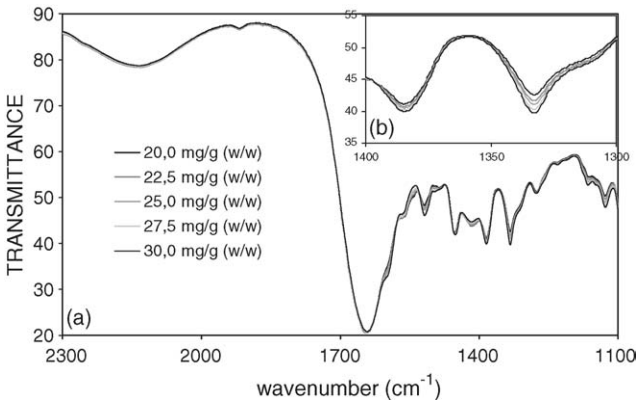

Fig. 2. Overlay of spectra of five reconstituted gels: (a) 1100–2300 cm^{-1} and (b) 1300–1400 cm^{-1}.

The PLS method was validated before its application for quantitative determination of NFA in commercial samples of NIFLUGEL®.

The linearity of the PLS model was established with the linear regression equation obtained with the least square method (Fig. 4) applied to introduced mass concentration (IMC) and predicted mass concentration (PMC) values for the validation set:

$$\mathrm{PMC\,(mg\,g^{-1})} = 1.0093(\pm 0.0099)\,\mathrm{IMC\,(mg\,g^{-1})} \pm 0.4853(\pm 0.2486) \quad (1)$$

with $R = 0.9988$ and $F = 10483$.

As R is close to 1 and F highly significant [F (0.05, 1, 24) = 4.24], the linearity of the method is demonstrated.

The specificity of the PLS method was determined by testing the significance of the y-intercept of the linear model. As Student's t-test shows that the y-intercept of the regression

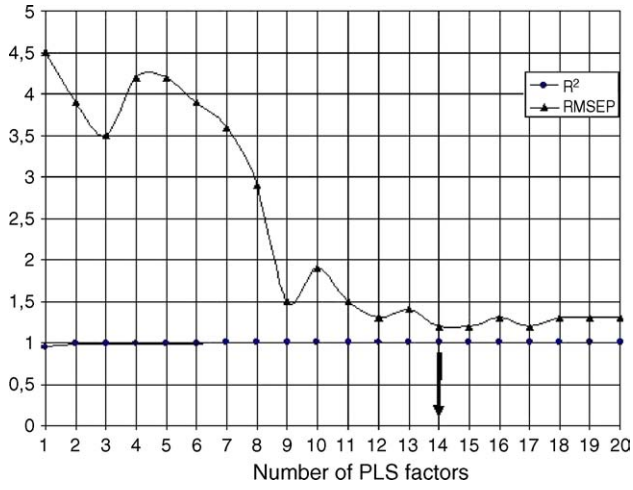

Fig. 3. Determination of the number of significant factors of PLS model.

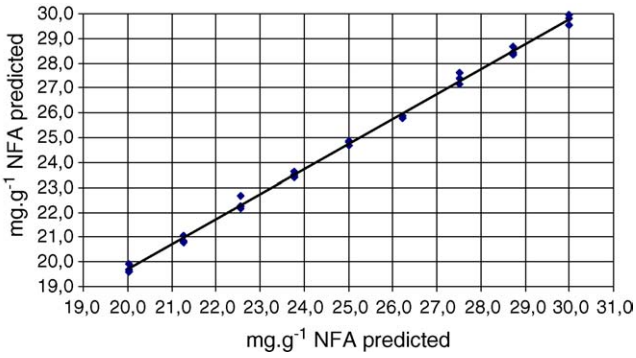

Fig. 4. Predicted versus introduced mass concentration of NFA (mg g^{-1}) of a series of gels containing 20–30 mg g^{-1} of NFA (model with 14 PLS factors).

Eq. (1) is not significantly different from zero [$t = 1.95 < t^{\circ}(0.05, 25) = 2.09$], the specificity of the method is confirmed.

The accuracy was expressed as mean value of recovery percentage with 27 values of the validation set. The mean percentage of recoveries was found to be 101.06 (±0.72) and the relative standard deviation (R.S.D.) was 0.71%, indicating a very good accuracy (Table 1). Furthermore, the accuracy was found relatively similar along the full range of tested concentration of the model. No previous methods led to so high accuracy. With HPLC [4], the value was near 5%. Valuable value was obtained with capillary isotachophoretic [3], 1.55% was reached, however the analysis time was 20 min.

The precision of the method was established with six reconstituted gels at 25 mg g^{-1} of NFA. The repeatability (or intra-day precision) and the reproducibility (or inter-day precision) were expressed as the R.S.D. of the mean percentage recovery of NFA determinations performed on the same day and on three different days, respectively. The both were found (Table 2) smaller than the standard value from ICH guideline [24].

The limit of detection of the method was calculated according to ICH recommendation [24]. It was based on the standard deviation of the response and the slope and was equal to 1.29 mg g^{-1}.

This optimised calibration model was used to determine the NFA amount of six commercial samples of NIFLUGEL® (Table 3). These results show the absence of significant difference between the theoretical and calculated concentrations for each tested sample.

Table 1
Percentage of recovery of niflumic acid ($n = 27$)

Introduced mass concentration of NFA (mg g^{-1})	Predicted mass concentration of NFA (mg g^{-1})	Recovery (%)
20.03	19.69	101.72
20.03	19.59	102.21
20.03	19.92	100.52
21.27	20.80	102.27
21.27	21.07	100.92
21.27	20.84	102.03
22.56	22.66	99.53
22.56	22.18	101.71
22.56	22.26	101.36
23.77	23.41	101.54
23.77	23.50	101.13
23.77	23.62	100.63
25.00	24.88	100.50
25.00	24.81	100.75
25.00	24.69	101.27
26.23	25.80	101.66
26.23	25.89	101.30
26.23	25.88	101.36
27.51	27.61	99.63
27.51	27.17	101.26
27.51	27.41	100.38
28.73	28.67	100.21
28.73	28.33	101.41
28.73	28.42	101.10
30.00	29.83	100.57
30.00	29.56	101.49
30.00	29.98	100.07
Mean recovery		101.06
R.S.D.		0.71

Table 2
Precision of PLS model

	Intra-day precision ($n = 6$)		Inter-day precision ($n = 18$)	
	Calculated values	Standard value	Calculated values	Standard value
Mean percentage recovery	100.66		99.46	
R.S.D. (%)	0.65	<2	1.58	<3

Table 3
Quantitative determination of NFA for six commercial gels of NIFLUGEL®

NIFLUGEL®	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Predicted mass concentration of NFA (mg g^{-1})	24.62	24.15	25.26	24.67	24.42	24.51
Recovery (%)	98.49	96.60	101.02	98.67	97.68	98.05
Mean value (%)			98.42			
R.S.D. (%)			1.36			

4. Conclusions

ATR/FTIR together with PLS provides high-performance approach for NFA quantitave determination in order to check the label-claimed content in pharmaceutical formulation of NIFLUGEL®. This method is rapid, non-destructive and easy-to-use. The analytical procedure requires no sample pre-treatment and reagent-free measurement. Great precision of method is a requirement of quality control in pharmaceutical industry and is an advantage in this case of formulation containing such rapid-absorption drug. This procedure constitues a powerful alternative to separative method for quality control analysis of pharmaceuticals.

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