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Short communication

The influence of sample area on diclofenac sodium quantification by diffuse reflectance IR spectroscopy

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ABSTRACT

A procedure for the quantitative determination of diclofenac sodium (DS) in commercial capsules and tablets based on Partial Least Squares (PLS) treatment of diffuse reflectance FTIR spectroscopic (DRIFTS) data is described. Two DRIFTS accessories, a Collector II (Spectra-Tech) and a Seagull (Harrick Scientific), were used to collect the spectra. The spectrometer beam area on the surface of the sample was approximately sevenfold smaller for the Collector II accessory compared to the Seagull accessory. Spectra collection using the smaller beam spot resulted in significantly higher quantification errors for the single measurements. To reduce the errors associated with the Collector II accessory spectra were collected seven times while randomly changing the sample position. The mean spectra were used in the analysis. To compare the predictive ability of the constructed models, the relative standard errors of prediction (RSEP) were calculated. The RSEPs were 1.3–2.9% and 2.0–2.6% using the Collector II accessory and 1.0–1.5% and 1.1–1.7% using the Seagull accessory, for calibration and validation data sets, for the different PLS models. Three commercial preparations containing 20.5, 23.2 and 34.5% DS were successfully quantified using the developed models. The proposed procedure can be used as a fast, precise and economic method for DS quantification in tablets and capsules.

1. Introduction

A number of analytical techniques are used for the quantification of active ingredients in pharmaceuticals. The first step of the analysis often includes dissolution of the compound of interest. This step increases the analysis time and makes it more expensive. Fortunately, there are techniques available that can be used for the quantification of active pharmaceutical ingredients in different dosage forms without the need for solvation or extraction and that require only minimal pre-treatment of the analyzed sample. Among them near infrared (NIR) and Raman spectroscopy ought to be mentioned as the most commonly used. One of these techniques, less popular than the previous two, is diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) [1,2]. DRIFTS is a low-cost technique that has gained popularity in the last few decades due to its simplicity. Now DRIFTS is commonly used for the qualitative analysis and identification of solid pharmaceutical ingredients [3,4]. DRIFTS spectroscopy in conjunction with chemometric methods of data treatment can be a useful tool in quantification of solid mixtures, including pharmaceuticals [5–14]. However, it seems that a wider adoption of the technique to quantitative analysis has been hampered by some factors (e.g. the particle size and shape, properties of the sample surface and the heterogeneity of the sample), that influence spectral reproducibility and are responsible for typical quantification errors [5]. To reduce these errors, it is common to average spectra that are collected from several different places on the sample surface or are collected from several repacks of the same sample [5,6]. Another possibility of signal averaging is similar to that one which has proven to be very effective for Raman spectroscopy [15,16]. If one collects Raman spectra using a rotating sample holder, the area of the sample that is illuminated during the experiment is increased, usually by one to two orders of magnitude, in comparison to the area probed when using a standard, non-moving sample holder. Rotating a powder sample in DRIFTS is more difficult than rotating a typical solid Raman sample, but there are other ways to increase the active area of the analyzed sample. In our experiments, we used two DRIFTS attachments that probe sample areas that differ six- to eight-fold in size depending on the alignment. These accessories include the Collector II, which strongly condenses spectrometer beam, and the versatile Seagull attachment, in diffuse reflection mode, which is less effective than the former accessory but collects signal from a noticeably larger sample area.

Diclofenac sodium, a sodium salt of 2-[(2,6-dichlorophenyl)aminophenyl]-acetic acid is known as a potent analgesic and anti-inflammatory agent, commonly used in various drug formulations, including tablets, capsules, drops, injections,

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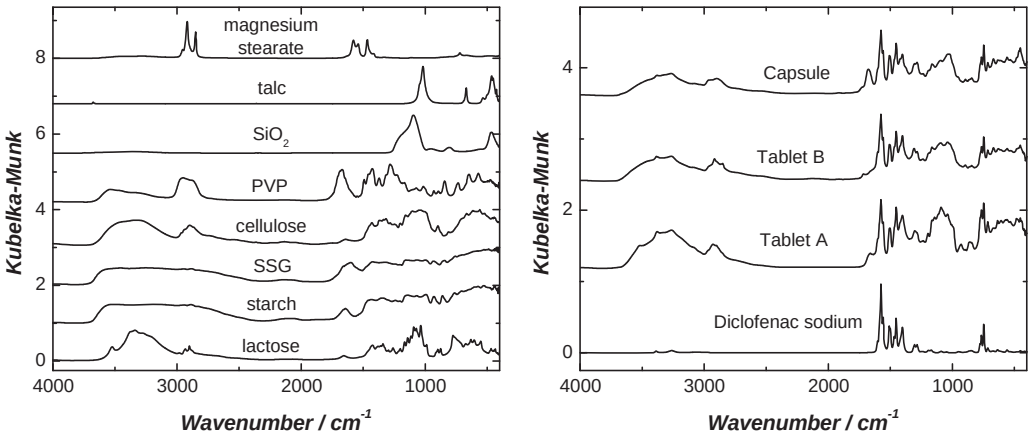

Fig. 1. DRIFTS spectra of excipients (left), diclofenac sodium and its analyzed preparations (right); the spectra are offset for clarity; PVP, polyvinylpyrrolidone; SSG, sodium starch glycolate.

suppositories and gels [17]. Several analytical methods of DS quantification in pharmaceuticals have been developed. Among them the UV–vis spectrometry, liquid chromatography, high performance liquid chromatography and potentiometry or spectrofluorometry can be listed as the most popular [18], although less common techniques were also proposed [19]. The suitability of Raman spectroscopy application to its quantitative analysis in tablets, capsules and injection solutions was demonstrated [18,20].

Composition of the studied pharmaceuticals was determined at the beginning. Lactose, starch, cellulose, sodium starch glycolate, SiO_2, magnesium stearate and polyvinylpyrrolidone were found as additives in the studied tablets. Cellulose, SiO_2, talc and eudragit were detected as the main excipients in the studied capsules.

Herein, we present the results of diclofenac sodium (DS) quantification in commercial tablets and capsules obtained by Partial Least Squares (PLS) treatment of DRIFTS data. Comparing results collected using the two DRIFTS accessories, we demonstrate that the size of the sample area probed during an experiment is a cardinal parameter determining the robustness of the models and the quality of quantification.

2. Experimental

2.1. Apparatus

A Nicolet Magna 860 FT-IR spectrometer equipped with a KBr beamsplitter and DTGS detector was used to carry out the measurements. The interferograms were averaged over 64 or 128 scans, Happ-Genzel apodized and Fourier transformed using a zero filling factor of 2 to give spectra in the 400–4000 cm^{-1} range at the resolution of 4 cm^{-1}. It took approximately 1–2 min to obtain a spectrum in these conditions. Spectra of each sample were collected twice using both the Collector II and the Seagull DRIFTS accessories. The sample was placed in the sample cup and leveled with a metal edge. The beam spot area on the surface of the sample was approximately seven times bigger for the Seagull accessory compared to the Collector II accessory. To reduce quantification errors when using the Collector II attachment, it was necessary to record spectra for the same sample a few times at randomly rotated sample positions. For each sample, seven spectra were collected with the Collector II accessory, and the mean spectrum was utilized for the analysis.

2.2. Materials and sample preparation

The substances used, namely diclofenac sodium, cellulose, lactose, starch, talc, eudragit and polyvinylpyrrolidone were of pharmacopoeial purity. Sodium starch glycolate, magnesium stearate and SiO_2 were of an analytical grade.

Three preparations of DS: tablets A and B containing a declared 50 mg of API per tablet and capsules C containing a declared 75 mg of API per capsule were purchased in a local pharmacy.

Samples with the suitable weight ratios of compounds were prepared by mixing pure, solid substances in a mortar for a few minutes, to homogenise powders properly. Then, approximately 100 mg of powder was diluted with the appropriate amount of dried and ground KBr to obtain a desired level of the Kubelka-Munk DRIFTS signal (Fig. 1). The commercial preparations were processed in a similar way.

To avoid collinearity between concentrations of components of the studied mixtures concentration versus concentrations graphs were plotted. No significant correlations were observed. The determination coefficients R^2 for these plots changed in the 0.01–0.19 range.

2.3. Chemometric models

Nicolet TQ Analyst ver. 7.0 chemometric software was used to construct calibration models and to perform the quantitative analysis of active pharmaceutical ingredient (API) in commercial products. Spectral data were mean-centred.

To characterise the prediction ability of elaborated models the relative standard error of prediction (RSEP) was calculated according to [21]:

$$\mathrm{RSEP}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_i - C_i^A)^2}{\sum_{i=1}^{n} C_i^{A2}}} \times 100, \quad (1)$$

in which C^A is the actual component content, C is the concentration found from DRIFTS data analysis, and n is the number of samples. The predicted residual sum of squares (PRESS) was calculated to select an optimal number of factors for PLS models.

To estimate the performance of the constructed models the cross-validation technique, leave-one-out, was applied. A correlation coefficient R_{cv} characterising a plot of computed versus actual concentration for each sample removed in turn from the data set and quantified on the basis of model obtained without this sample was determined.

3. Results and discussion

Separate calibration mixtures were prepared for capsules and tablets, both containing DS as the API. The quantitative compo-

Table 1
Calibration parameters for diclofenac sodium determination for SNV normalised DRIFT spectra.

Parameter	Capsules		Tablets A		Tablets B	
	DRIFTS accessory		DRIFTS accessory		DRIFTS accessory	
	Collector	Seagull	Collector	Seagull	Collector	Seagull
Number of samples	30	30	35	35	35	35
$RSEP_{calibration}$ [%]	2.95	1.51	1.28	1.17	1.95	1.03
$RSEP_{validation}$ [%]	2.58	1.67	1.96	1.12	2.00	1.14
R	0.964	0.991	0.998	0.999	0.983	0.999
R_{cv}	0.928	0.963	0.995	0.991	0.962	0.993

sition of the studied mixtures was expressed as a mass fraction. For capsule analysis, the mass fraction varied between 0.25–0.37, 0.29–0.44, 0.20–0.33, 0.03–0.10 and 0.02–0.07 for DS, cellulose, SiO_2, eudragit and talc, respectively. For mixtures modeling tablet composition, the mass fraction varied between 0.10–0.26, 0.20–0.30, 0.11–0.20, 0.11–0.19, 0.07–0.15, 0.04–0.09, 0.04–0.08 and 0.04–0.08 for DS, starch, cellulose, lactose, sodium starch glycolate, polyvinylpyrrolidone, SiO_2 and magnesium stearate, respectively. The conformity of the composition of the prepared sets of mixtures and of the analyzed preparations was controlled using the Principal Component Analysis method. No outliers were detected, at 99% confidence level, using the score plots for the first 3 components.

DRIFTS spectra were collected for 30 or 35 samples (Table 1), and PLS calibration models were built using standard normal variate (SNV) normalized spectra [22]. In some cases, the mean value normalization (MVN) procedure [23]:

$$I_i' = \frac{I_i}{I_{\text{mean}}}, \tag{2}$$

gave smaller quantification errors; I_i is not normalized, I_i' is normalized, I_{mean} is the mean value of the spectral density.

In Fig. 1, the DRIFTS spectra of the analyzed commercial preparations and constituents of the studied tablets and capsules are presented. The compositions of tablets A and B were similar, which resulted in similar vibrational spectra.

Six mixtures were chosen for the validation of each system studied, and the remaining samples were used as a training set. For mixtures modeling capsule composition, three spectral ranges, 410–475, 735–790 and 1465–1690 cm^{-1}, were used to construct the PLS models. For modeling tablet composition, two spectral ranges, 410–1690 and 2620–3680 cm^{-1} were used.

A significant difference in the quality of the elaborated models was observed (Table 1). For the three studied preparations, models constructed from data collected with the Seagull attachment were more robust and precise than those obtained using spectra collected with the Collector II accessory. This conclusion was inferred from the $RSEP_{calibration}/RSEP_{validation}$ values, which are 1.51/1.67% (2.95/2.58%), 1.17/1.12% (1.28/1.96%) and 1.03/1.14% (1.95/2.00%)

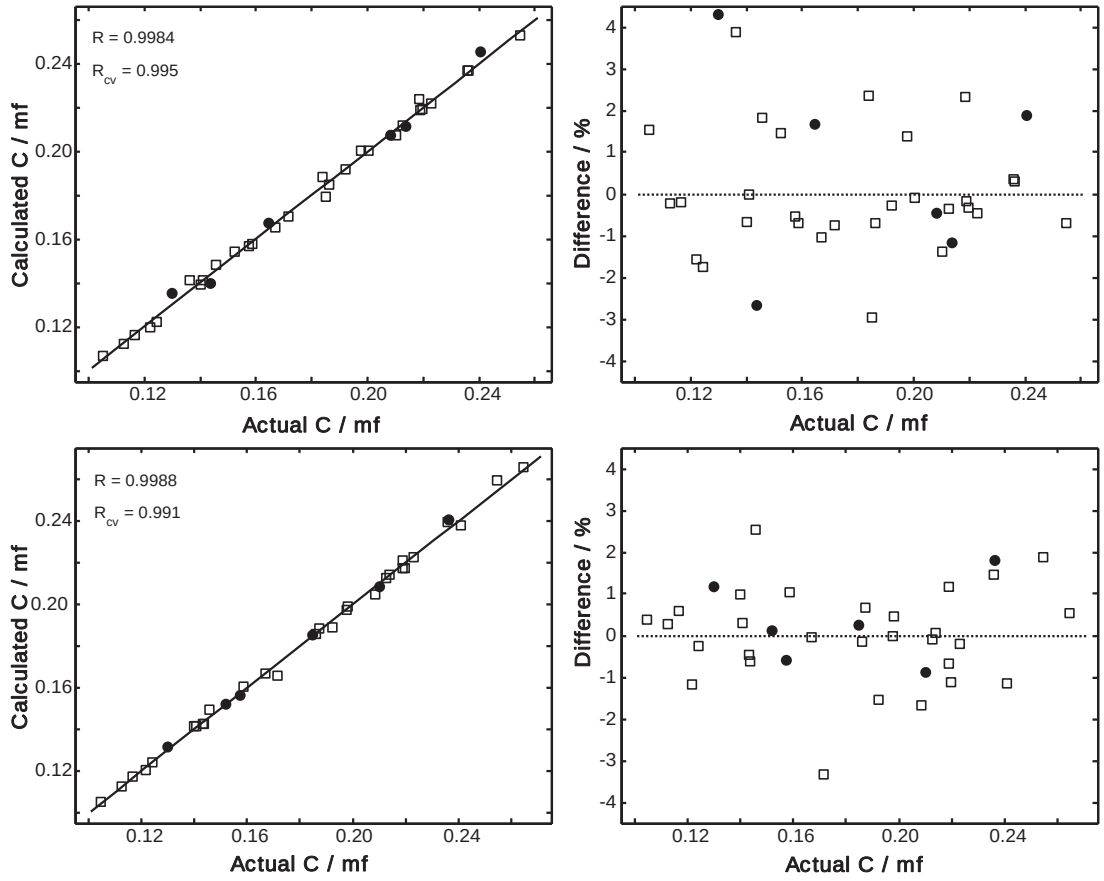

Fig. 2. Calibration curves and relative errors for diclofenac sodium content obtained using the PLS models for tablets A based on the Collector II (top) and the Seagull (bottom) spectra.

Table 2
Results (in milligrams) of DRIFTS analysis of the studied preparations ($n = 7$).

Preparation (declared content)	SNV normalised		MVN normalised		Reference analysis[a]
	DRIFTS accessory		DRIFTS accessory		
	Collector	Seagull	Collector	Seagull	
Capsules (75)	74.94 ± 1.47	75.04 ± 0.87	75.05 ± 1.18	75.07 ± 0.95	75.61 ± 0.62
Tablets A (50)	49.94 ± 0.81	49.99 ± 1.29	49.91 ± 0.89	49.97 ± 0.93	49.11 ± 0.74
Tablets B (50)	49.93 ± 0.50	50.02 ± 0.77	50.03 ± 0.85	50.11 ± 0.47	50.04 ± 1.15

[a] Taken from Ref. [18].

for capsules, tablets A and tablets B, respectively. The errors shown in parentheses are for the models based on spectra collected with the Collector II accessory. Five or six PLS factors were necessary for reliable model construction. They accounted for 98–99% of spectral variation in the studied systems. The correlation coefficients of the calibration curves for API modeling were between 0.991–0.999 (0.964–0.998), and the R_{cv} parameters were between 0.96–0.99 (0.93–0.99). These values are higher for the Seagull data compared to the Collector II data that is shown in parentheses. The calibration curves and plots of the relative errors for DS quantification in tablets A are shown in Fig. 2.

Using the described models, the studied pharmaceuticals were quantified. The mean concentrations of API found using the DRIFTS spectra agree excellently with those obtained from the reference analysis (Table 2). Quantification of the preparations resulted in DS recoveries between 99.2–101.8% (99.9–100.2%) when using the Seagull accessory and 99.1–101.7% (99.8–100.1%) when using the Collector II accessory. The recoveries calculated against the declared API content are provided in parentheses.

PLS models based on single spectra recorded for each sample with the Collector II accessory had unacceptably high RSEP error values. They were two to three times higher than those errors found when mean spectra ($n = 7$) were used. As a result, for these models, noticeably larger quantification errors were observed.

Non-uniform distribution of API and excipients in solid formulations is well documented [3,24]. The presented results confirm that in quantitative analysis of solid multi-component mixtures, it is essential to properly take the heterogeneity of the sample into account. In some experiments, careful grinding of samples can help to reduce this heterogeneity problem. Nevertheless, signal collection from a large enough area of the analyzed sample is still of vital importance for reliable API quantification.

4. Conclusions

This study confirms the high potential of DRIFTS combined with the PLS algorithm for the quantitative analysis of APIs in solid dosage forms of pharmaceuticals. The described method is fast, simple and inexpensive. Except for grinding, this method does not require special preparations of the capsules or tablets to be analyzed.

The accuracy and precision of the proposed procedure is comparable to other analytical techniques that are routinely used for this purpose. Quantification of the analyzed preparations gave a DS content with recoveries between 99.1 and 101.8% for the different models constructed.

The area of the sample probed during the experiment was shown to be a crucial factor that influences the quality of the obtained results. This suggests that DRIFTS accessories dedicated for quantitative applications should illuminate as large part of the sample surface as possible to average spectroscopic signal over the probed area.

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