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Quantification of active ingredients in suppositories by FT-Raman spectroscopy

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An efficient method for the quantitative determination of acetaminophen (AAP) and diclofenac sodium (DS) in commercial suppositories based on partial least squares (PLS) treatment of FT-Raman spectra is described. The relative standard errors of prediction (RSEP) were calculated for calibration and validation data sets to evaluate the quality of the constructed models. In the case of DS determination, RSEP error values of 1.9 % and 2.3 % for the calibration and validation data sets, respectively, were found. For AAP these errors amounted to 1.6–2.3 % and 1.8–2.8 %, respectively, for the different calibration models. Four commercial preparations containing 5, 12.5, 16.7 and 33.3 % (*w/w*) AAP and one containing 5 % (*w/w*) DS were successfully quantified using the developed models. Concentrations derived from the developed models correlated strongly with the declared values and yielded recoveries of 99.4–100.2 % and 99.6 % for AAP and DS, respectively. The proposed procedure can be used as a fast, economic and reliable method for quantification of the active pharmaceutical ingredients in suppositories.

Keywords: paracetamol; diclofenac sodium; suppository; Raman spectroscopy; partial least squares; quantitative analysis

Introduction

Acetaminophen (AAP) or paracetamol (*N*-(4-hydroxyphenyl)acetamide) and diclofenac sodium (2-[(2,6-dichlorophenyl)aminophenyl]-acetic acid sodium salt; DS) are analgesic and anti-inflammatory active compounds.[1,2] The former is also an antipyretic agent. They are active pharmaceutical ingredients (APIs) in a variety of drug formulations, which include tablets, capsules, suppositories, and injection solutions as well as suspensions in the case of AAP and gels and drops in the case of DS. Paracetamol is the most commonly used over-the-counter medicine worldwide.[3] Various techniques, such as UV-VIS spectrometry, spectrofluorometry, liquid chromatography, densitometry, potentiometry and Raman spectroscopy, have been implemented to quantify these APIs.[4–6]

Limitations with the oral route of drug administration, such as gastrointestinal side effects, vomiting, and uncooperative patients prompted the need for the development of suppository formulations for a number of active substances. Suppositories are made from a greasy or water-soluble base in which the active ingredient is dissolved or suspended. In the case of AAP and DS suppositories, the API crosses the rectal mucosa into the bloodstream.

Raman spectroscopy has become an increasingly important tool in the quantitative analysis of complex mixtures,[7] yielding reliable results for tablets, capsules, and solutions.[5,6] Unlike many analytical techniques, this method usually does not require the dissolution or extraction of the samples being analyzed, which substantially simplifies and shortens the analysis. To the best of our knowledge, there are no examples of the application of Raman spectroscopy to the quantification of APIs in suppositories.

The most popular chemometric tool used for modelling multidimensional quantitative spectroscopic data is a partial least squares (PLS) regression.[8] In this technique, a regression model that establishes a linear relationship between the dependent variables Y (concentrations of the components in this analysis) and the independent variables X (sample Raman spectra in these experiments) is calculated. PLS involves the calculation of new variables which are linear combinations of the original variables. They are obtained in such a way that the variance in the X variables they explain decreases from the first to the last variable and they are orthogonal. The PLS method calculates factors that maximize the amount of variation explained in the independent variable matrix that is relevant for predicting the dependent variable matrix.[8]

The main objective of this study was to investigate the feasibility of using FT-Raman spectroscopy along with PLS regression for the fast and precise quantification of the active component in commercial suppositories containing 5% (*w/w*) of DS or 5–33% (*w/w*) of AAP.

Experimental

Materials and sample preparation

Pharmacopoeial purity paracetamol (Galena, Wroclaw, Poland) and suppository base Estaram H15 as well as diclofenac sodium (>98%) from Sigma-Aldrich (St.Louis, MO, USA) were used as received. Five preparations were purchased from a local pharmacy: A contained a declared amount of 50 mg of DS per suppository and B, C, D, and E contained declared amounts of 50, 125, 250, and 500 mg, respectively, of AAP per suppository. Suppositories A, B, and C weighed 1 g, whereas D and E weighed 1.5 g which means that declared concentration of the API was equal to 5.0, 5.0, 12.5, 16.7, and 33.3% (*w/w*) in preparations A, B, C, D, and E, respectively.

Samples with suitable weight ratios of compounds were prepared by mixing the pure, solid substances in a mortar for a few minutes to homogenize them properly. The mixture was then

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Table 1. API content in the studied mixtures [%].

Sample	Diclofenac sodium	Acetaminophen
1	3.27	4.08
2	3.70	6.55
3	4.25[a]	11.49
4	4.59	13.97[a]
5	4.95	16.47
6	5.40	18.93
7	5.78[a]	21.44
8	6.26	24.06[a]
9	6.56	26.56
10	7.09	29.01
11	7.35[a]	31.49[a]
12	7.87	33.98
13	8.15	36.50
14	8.58	39.02[a]
15	8.96[a]	41.52
16	9.31	44.08
17	9.77	46.43
18	10.22[a]	49.14[b]
19	10.54	51.61[a]
20	10.99	54.11
21	11.40	56.42
22	11.92[b]	59.37

[a]–validation sample
[b]–ignored sample

delicately heated using hot air until melting occurred, and the sample was transferred to a glass tube. The samples were then cooled to room temperature. The commercial preparations were processed in a similar way. As the calibration samples were composed of the API and suppository base only, it was not possible to avoid collinearity between concentrations of components. We neglected preservatives present in suppositories in minute amounts.

To construct calibration models, the FT-Raman spectra of 22 samples were used. The mass fraction of the API varied within the 0.03–0.12 range for mixtures modeling DS-containing suppositories and within the 0.04–0.59 range for mixtures modeling AAP-containing suppositories (Table 1).

Apparatus and numerical data treatment

A Nicolet Magna 860 FT-IR spectrometer interfaced with an FT-Raman accessory was used to record the spectra at a resolution of 16 cm^{-1}. The samples placed in a glass tube of 5 mm internal diameter were rotated at a speed of approximately 150 rpm. They were illuminated by an Nd:YVO4 laser line at 1.064 μm without a converging lens. A CaF_2 beamsplitter and an indium gallium arsenide detector were used. The interferograms were averaged over 256 scans, Happ-Genzel apodized and Fourier transformed using a zero filling factor of 2 to give spectra in the 100–3700 cm^{-1} range. Spectra were recorded with a power of 250 mW at the sample. PLS models were constructed with Nicolet TQ Analyst v.7 chemometrics software. The principal component analysis (PCA) was performed using PLS_Toolbox v.4 in the Matlab environment.[9] The relative standard errors of prediction, RSEP, were calculated for the calibration and validation data sets.[5]

Results and discussion

In Figure 1, the FT-Raman spectra of pure AAP, pure DS, and the studied commercial preparations are presented. All five analyzed medicines contain an active component, 5.0% (*w/w*) of DS and 5.0, 12.5, 16.7, and 33.3% (*w/w*) of AAP, and Estaram as the suppository base.

The conformity of the composition of the prepared sets of mixtures and the analyzed suppositories was controlled using the PCA method. The PLS calibration model for DS medicines was built using standard normal variate (SNV) normalized spectra.[10] In the case of AAP suppositories, a multiplicative scatter correction procedure (MSC) yielded smaller quantification errors.[11] Based on the predicted residual error sum of squares plots, calibration models were build using three PLS factors.

Five mixtures were chosen for the validation procedure for each system. The remaining samples were used as a training set, except for one DS sample and one AAP sample which were treated as outliers (Table 1).

As was mentioned before, calibration mixtures contained only major components present in the studied suppositories so it was necessary to select appropriate spectral ranges for the analysis. This was done using PCA and scores plots. Two ranges of Raman spectra were applied in PLS model construction: 198–1810 and 2495–3097 cm^{-1} for DS, and 190–1820, and 2400–3375 cm^{-1} for AAP suppositories, which means

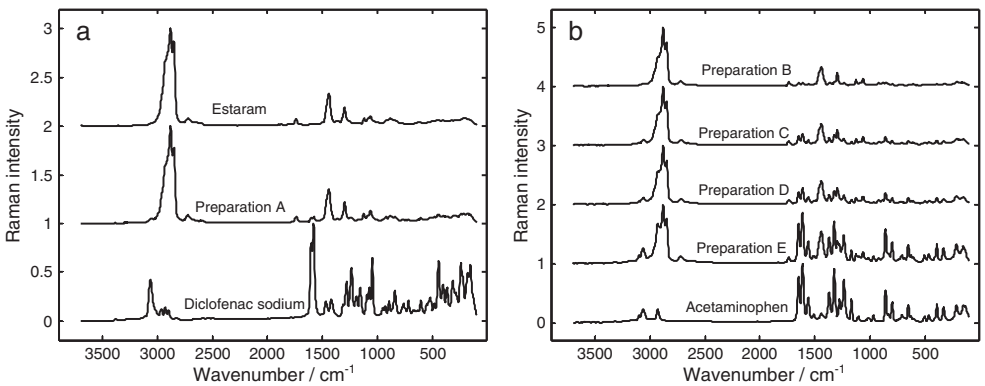

Figure 1. FT-Raman spectra of diclofenac sodium (a) and acetaminophen (b) with the spectra of their analyzed preparations and the spectrum of Estaram; the spectra are offset for clarity.

Table 2. Parameters of the PLS models.

Parameter	Diclofenac sodium	Acetaminophen[a]
R	0.9983	0.9991
$RSEP_{cal}$ [%]	1.88	2.03
$RSEP_{val}$ [%]	2.26	1.84
R_{cv}	0.995	0.993

[a]–model optimized for preparation C

Table 3. Results [%] of FT-Raman analysis of the studied suppositories (n = 8).

Preparation	Declared content	Determined content	Recovery
A	5.0	4.98 ± 0.10[a]	99.6
B	5.0	4.98 ± 0.17	99.6
C	12.5	12.48 ± 0.29	99.8
D	16.7	16.73 ± 0.29	100.2
E	33.3	33.11 ± 0.38	99.4

[a]–standard deviation

that mainly parts of the spectra without pronounced Raman features were excluded. These regions were modified for the each studied AAP preparation which resulted in a change of $RSEP_{cal}$ and $RSEP_{val}$ parameter values in the 1.6–2.3% and 1.8–2.8% range, respectively, and in lower quantification errors for the real samples. There is no noticeable difference in the quality of the models for the two APIs that were quantified (Table 2). For DS suppositories, the $RSEP_{cal}$/$RSEP_{val}$ is found to be 1.9/2.3%. The correlation coefficient, R, for obtained calibration curves changes between 0.9983 and 0.9992. The developed models are characterized by a high resistance to the leave-one-out cross-validation procedure, yielding comparable R_{cv} parameter values (>0.993). The calibration curves and plots of the relative errors for the quantification of DS and AAP in suppositories are shown in Figure 2; in the case of AAP, the model optimized for preparation C is presented.

Using the described models, five commercial suppositories were quantified. The mean concentrations of API found using Raman spectral analysis agree very well with the declared values (Table 3). Quantification of the studied preparations resulted in AAP recoveries between 99.4 and100.2%, and 99.6% for DS. The recoveries were calculated against the declared API content.

Conclusions

Four preparations containing 5.0, 12.5, 16.7, and 33.3% (*w/w*) of AAP and one medicine containing 5.0% (*w/w*) of DS were successfully quantified using PLS models based on FT-Raman spectra. Concentrations of the API found from the developed models correlated strongly with the declared content.

The described method is reliable, fast, and inexpensive. It does not require any preparation of the suppositories to be analyzed.

Acknowledgment

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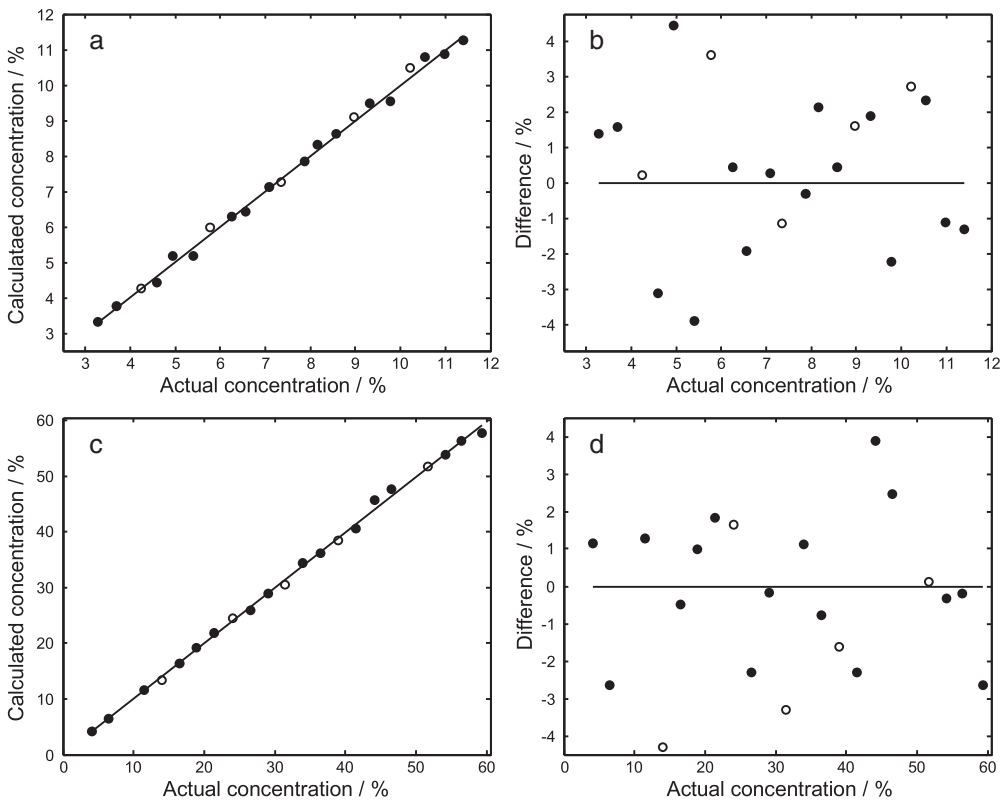

Figure 2. Calibration curves and relative errors of the API content obtained using the PLS models for diclofenac sodium (a/b) and acetaminophen suppositories (c/d); filled symbols, calibration; open symbol, validation data sets.

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