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Short communication

Quantitative determination of captopril and prednisolone in tablets by FT-Raman spectroscopy

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Abstract

A procedure for the quantitative determination of captopril and prednisolone in commercial tablets based on partial least squares (PLS) and principal component regression (PCR) treatment of FT-Raman spectroscopic data is described. In the studied medicines active pharmaceutical ingredients (APIs) constitute 4.2–16.7% of the tablet mass. Results obtained from calibration models built using unnormalised spectra were compared with the values found when an internal standard was added to each sample and the spectra were normalised by its selected band intensity at maximum or integrated. To apprise the quality of the models the relative standard error of predictions (RSEPs) were calculated for calibration and testing data sets. For captopril determination these were 1.8–2.2% (2.1–2.3%) and 2.7–3.1% (2.7–3.6%), respectively for the different PLS (PCR) models. For prednisolone these errors amounted to 1.8–2.1% (2.6–3.5%) and 3.2–3.7% (3.7–5.9%), respectively. Three commercial preparations of captopril containing 12.5 mg and one 25 mg of API per tablet were quantified using developed models. Found captopril contents, calculated versus results of iodometric titration, was equal 99.2–101.2% (99.2–102.0%), for the different PLS (PCR) calibration models and the different preparations. Quantification of prednisolone tablets, declared content 5 mg per tablet, on the basis of PLS (PCR) models gave API amount, calculated versus results of UV–vis method, in the 99.0–101.0% (98.0–102.0%) range.
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Keywords: FT-Raman spectroscopy; Captopril; Prednisolone; Pharmaceuticals; Quantitative determination

1. Introduction

Captopril, (2S)-1-[(2S)-3-mercapto-2-methylpropanoyl]pyrrolidine-2-carboxylic acid is a specific inhibitor of the angiotensine converting enzyme which is widely used in the treatment of hypertension [1–3]. Several methods of captopril quantitative analysis in pharmaceutical preparations were reported. Among them quantification using iodometric titration [2], liquid chromatography (LC) [4], differential pulse polarography [5], flow-injection (FI) [6], chemiluminescence [7] also in connection with LC [8] or FI systems [9,10], fluorimetry [11], voltammetry [12], colorimetry and potentiometry [13] or biosensors [14] were described.

Prednisolone, 11-β,17,21-trihydroxypregna-1,4-diene-3,20-dione is a synthetic adrenal corticosteroid [1,2,15]. It has potent anti-inflammatory properties and is used in a wide variety of inflammatory conditions such as arthritis, colitis, asthma, bronchitis, certain skin rashes, and allergic or inflammatory conditions of the nose and eyes [16]. It was quantified using UV–vis spectrometry [15], chromatography [17] and chemiluminescence method [18].

Raman spectroscopy in conjunction with chemometric methods of data treatment can be a useful tool in quantification of complex mixtures, including pharmaceuticals [19]. Contrary to established opinion the influence of experimental conditions, difficult to control during Raman measurements, on the quality of complex mixture's quantification can be compensated to a large extent [20]. Unlike many other common analytical methods, this technique does not require any special sample preparation, so analysed drugs are usually in their unmodified forms. Often it is possible to determine active pharmaceutical ingredients (API) quantitatively in polymer blisters or in ampoules, what significantly simplifies the analysis [21,22].

In spite of these advantages, application of Raman spectroscopy to API quantification is not widespread. Quantitative

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Raman studies of the systems containing less than 10–20% of an active substance are especially scarce.

When all constituents of the analysed sample are known, e.g. during on-line process control in factories, calibration mixtures and analysed preparations have the same composition. Sometimes the detailed tablet composition is not known, however. In such a case it is possible to build a simplified calibration model based on samples containing only the studied active component and main tablet diluents. Based on this two–three component system the quantitative analysis of various preparations for the same active ingredient is possible [23,24].

In the present work the results of the quantification of commercial tablets containing ca. 7.8–16.7% of captopril and ca. 4.2% of prednisolone by FT-Raman spectroscopy are presented. Two techniques were applied. In the first one, multivariate calibration was performed on the basis of unnormalised spectra. In the second one, an internal standard was added to samples and spectra normalised by its selected band intensity were used to construct calibration models. The results found were compared with those obtained using reference pharmacopoeial procedures.

2. Experimental

2.1. Materials and sample preparation

The substances used, namely captopril, prednisolone, lactose, gelatine, starch and magnesium stearate, were of pharmacopoeial purity. Microcystalline cellulose and potassium ferrocyanide(II) trihydrate were of analytical grade. Three captopril preparations containing 12.5 mg and one 25 mg of API per tablet and one containing 5 mg of prednisolone per tablet 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. Approximately 200 mg of powder was used to prepare a pellet in a way similar to that used in IR spectroscopy. The commercial tablets were ground first and processed further alike calibration samples. In the second step, an appropriate amount of $K_4[Fe(CN_6)]\cdot 3H_2O$, chosen as an internal standard, was added to each sample. New pellets were prepared as described above and Raman spectra were registered again.

2.2. Apparatus

All spectra were recorded using a Nicolet Magna 860 FT-IR spectrometer interfaced with a FT-Raman accessory. A CaF_2 beamsplitter, an indium–gallium–arsenide (InGaAs) detector and 180° backscattering geometry were used. The samples placed in a rotating sample holder were illuminated by a Nd:YVO_4 laser line at 1.064 μm with a power of ca. 400 mW at the sample, without a converging lens. The interferograms were averaged over 512 scans, Happ-Genzel apodized and Fourier transformed using a zero filling factor of two to give spectra in the 100–3700 cm^{-1} range at the resolution of 8 cm^{-1}. Samples were rotated at a speed of ca. 200 rpm.

UV–vis measurements were performed using a Carry-5 Varian spectrometer.

2.3. Models

Nicolet TQ Analyst chemometric software was applied to construct partial least squares (PLS) and principal component regression (PCR) models and to perform the quantitative analysis of ingredients in commercial products. All spectral data were mean centred. The quantitative composition of the studied samples was expressed as a weight ratio for models constructed using spectra normalised by the 2091 cm^{-1} potassium ferrocyanide band intensity at maximum or integrated, or as a mass fraction for models based on unnormalised spectra.

To characterise the predictive ability of elaborated models the relative standard error of prediction (RSEP) was calculated according to the equation:

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

where C^{A} is the actual component content, C is the concentration found form Raman data analysis, and n is the number of samples.

3. Results

3.1. Captopril tablets

In Fig. 1 FT-Raman spectra of pure captopril and the commercial tablets are presented. All four analysed tablets, denoted A, B, C, and D, beside active component contain lactose, starch,

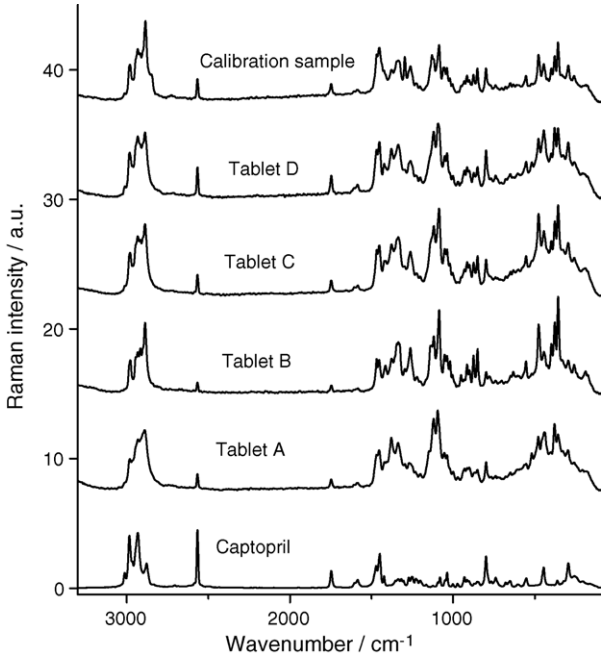

Fig. 1. FT-Raman spectra of pure captopril (bottom), analysed tablets (middle) and selected calibration mixture (top); Raman intensity of tablets and calibration sample multiplied by a factor five.

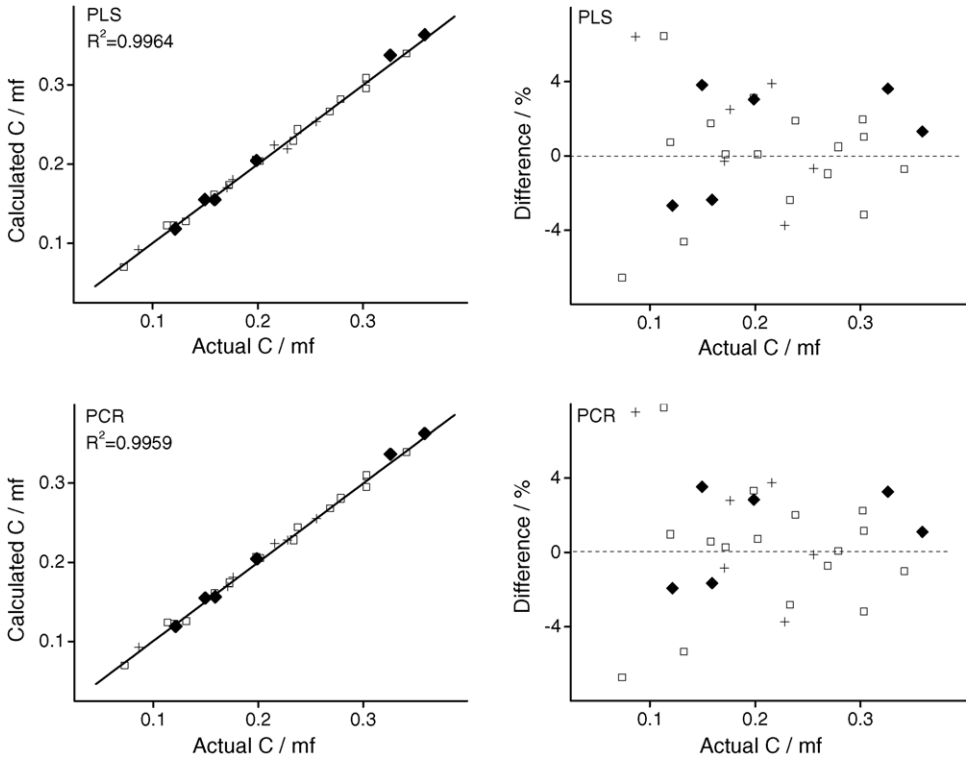

Fig. 2. Calibration curves and relative errors for captopril content obtained for PLS (top) and PCR (bottom) models based on unnormalised spectra; open symbols: calibration, plus: validation and filled symbols: testing data sets.

cellulose, and magnesium stearate in different proportions. The declared concentration, by weight, of captopril in these preparations is equal 7.8%, 12.4%, 12.8% and 16.7%.

To construct the calibration models spectra of 28 samples prepared as described above were used. Six mixtures were chosen for the validation procedure and six other were treated as "unknown" samples. The mass fraction varied in the 0.07–0.36 range for captopril, 0.09–0.42 for lactose, 0.09–0.33 for starch, 0.11–0.36 for microcrystalline cellulose and 0.04–0.16 range for magnesium stearate. To avoid the collinearity between concentrations of active compound and remaining constituents of the mixtures, concentration versus concentration graphs were plotted. No significant correlations were observed. The determination coefficient (R^2) varied in the 0.012–0.137 range for the active component and additives, and changed from 0.001 to 0.206 for remaining constituents.

The principal component analysis (PCA) shows that in such a complex system about 98% of the spectral variation could be accounted for by not less than five principal components. The following spectral ranges were applied in the chemometric models construction: 2940–2784, 2856–2482, 2002–1358, 1321–1182 and 1116–468 cm^{-1}. They were slightly modified for the each preparation studied. Typical calibration curves and relative differences calculated for the studied API are shown in Fig. 2. In Table 1 the RSEP values found for the calibration and testing samples quantification using PLS and PCR models are quoted. The RSEP error for captopril determination equals 2.7% for both models, while in the case of additives it changes from 1.9% for lactose to 6.5% for magnesium stearate.

Based on the same calibration models, the studied pharmaceuticals were quantified. The mean amount of captopril determined by FT-Raman analysis is collected in Table 2. These values show that both PLS and PCR approaches are comparably efficient in the case of testing samples. The mean concentration of captopril found, i.e. 12.5, 12.5, 12.5 and 25.2–25.3 mg correlate well with the results of reference analysis [2] which are presented in the last column of Table 2.

3.2. Captopril tablets, internal standard added

The quality of substance quantification by Raman spectroscopy depends on the full knowledge of the analysed system composition. If the content of calibration mixtures is close to analysed samples, one could expect more reliable results. But in such complex systems as pharmaceuticals, sometimes it is difficult to identify all ingredients present, especially if they constitute only a part of a percent of a studied sample mass. Analysis based on a simplified model usually results in an increase of

Table 1
RSEP (%) values for captopril determination

Samples	Unnormalised spectra		Normalised spectra			
			At maximum		Integrated	
	PLS	PCR	PLS	PCR	PLS	PCR
Calibration	1.82	2.19	2.12	2.33	2.18	2.13
Validation	2.71	2.56	2.87	3.04	2.92	2.85
Testing	2.75	2.74	3.15	3.58	3.12	3.38

Table 2
Results (in milligrams) of FT-Raman analysis of captopril tablets ($n = 5$)

Tablet	Declared contents	Raman analysis						Iodometric titration
		Unnormalised spectra		Normalised spectra				
				At maximum		Integrated		
		PLS	PCR	PLS	PCR	PLS	PCR	
A	12.5	12.5 ± 0.2	12.5 ± 0.2	12.6 ± 0.1	12.4 ± 0.2	12.5 ± 0.1	12.6 ± 0.2	12.5 ± 0.1
B	12.5	12.5 ± 0.2	12.5 ± 0.3	12.4 ± 0.1	12.5 ± 0.2	12.6 ± 0.2	12.4 ± 0.2	12.5 ± 0.1
C	12.5	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.2	12.5 ± 0.1
D	25	25.3 ± 0.3	25.2 ± 0.3	25.1 ± 0.5	25.1 ± 0.7	25.4 ± 0.5	25.6 ± 0.5	25.1 ± 0.2

quantification errors. An internal standard added could improve the quality of determination. This method works efficiently even when only the main components of a sample are known [23–25].

Following this route, potassium ferrocyanide was added as an internal standard to the mixtures and Raman spectra were recorded again. New PLS and PCR models were constructed using spectra normalised by the intensity at maximum and the integrated intensity of 2091 cm^{-1} ferrocyanide line.

For "unknown" samples the RSEP error value determined for the API quantification is in the range 3.1–3.6% for both models and both types of normalisation. Next, the commercial tablets with potassium ferrocyanide added were quantified. Mean content of captopril found in the studied tablets is presented in Table 2.

It is worth to notice that the quality of quantification of all four commercial captopril preparations based on the same PLS (PCR) model was only slightly worse than that one based on models modified for the each studied preparation. We obtained captopril contents, calculated versus results of pharmacopoeial method, in the range 99.2–101.6% (98.4–103.2%) and 99.2–101.2% (99.2–102.0%), respectively.

3.3. Prednisolone tablets

In an attempt to quantify even smaller concentrations of API than in the case of captopril, the analysis of prednisolone preparation was performed. Prednisolone is administered orally usually in doses of the order of a few milligrams per tablet. It means that an active ingredient usually amounts to only a few percent of the tablet weight. In Fig. 3 spectra of the pure substances which constitute the studied tablets together with the spectrum of the commercial pharmaceutical are shown. As one can notice, prednisolone spectrum is dominated by a strong peak of the ν_s(C=O) vibration at 1655 cm^{-1}. This isolated line could be easily recognised in prednisolone preparations even at relatively low active concentrations.

Lactose, starch, magnesium stearate and gelatine were found as additives in the studied prednisolone tablets. Calibration mixtures were prepared in a similar way to that described for captopril. The weight ratio varied in the range 0.02–0.12 for prednisolone, 0.19–0.74 for lactose, 0.13–0.66 for starch, 0.02–0.13 for magnesium stearate and 0.01–0.11 for gelatine. Next, Raman spectra of these mixtures were recorded. Based on these spectra the PLS and PCR models were constructed. The following

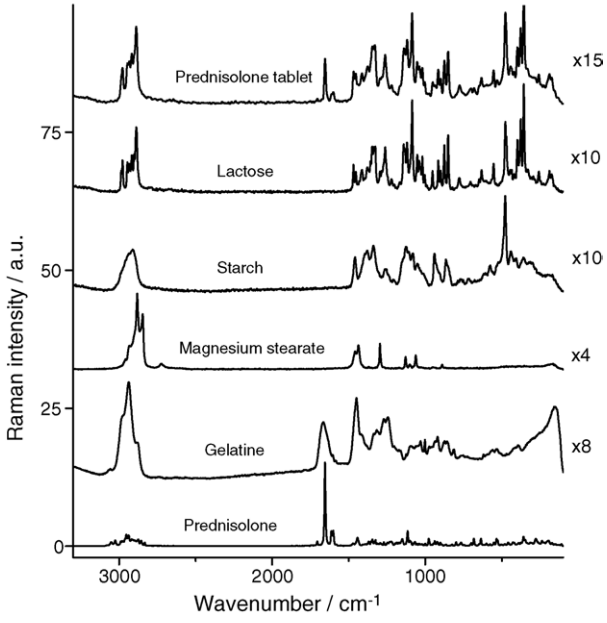

Fig. 3. FT-Raman spectra of analysed prednisolone preparation (top) and constituents of the studied tablets.

spectral ranges were applied in the described models construction: 3082–2818 (2818), 1897–1196 (1551), 1180–829 (968) and 658–217 (215) cm^{-1}. The baseline correction wavenumbers are quoted in the parenthesis. The obtained calibration curves for PLS models were characterised by R^2 values of the order of 0.990–0.995 (Fig. 4). The RSEP values calculated for prednisolone in the case of the testing sample set were ca. 3.2 and 3.7% for PLS and PCR methods, respectively. A full set of calibration results for prednisolone is quoted in Table 3. It is

Table 3
RSEP (%) values for prednisolone determination

Samples	Unnormalised spectra		Normalised spectra			
			At maximum		Integrated	
	PLS	PCR	PLS	PCR	PLS	PCR
Calibration	1.82	2.62	2.00	3.39	2.12	3.52
Validation	3.09	2.86	3.36	5.19	3.29	5.77
Testing	3.24	3.69	3.74	5.44	3.61	5.89

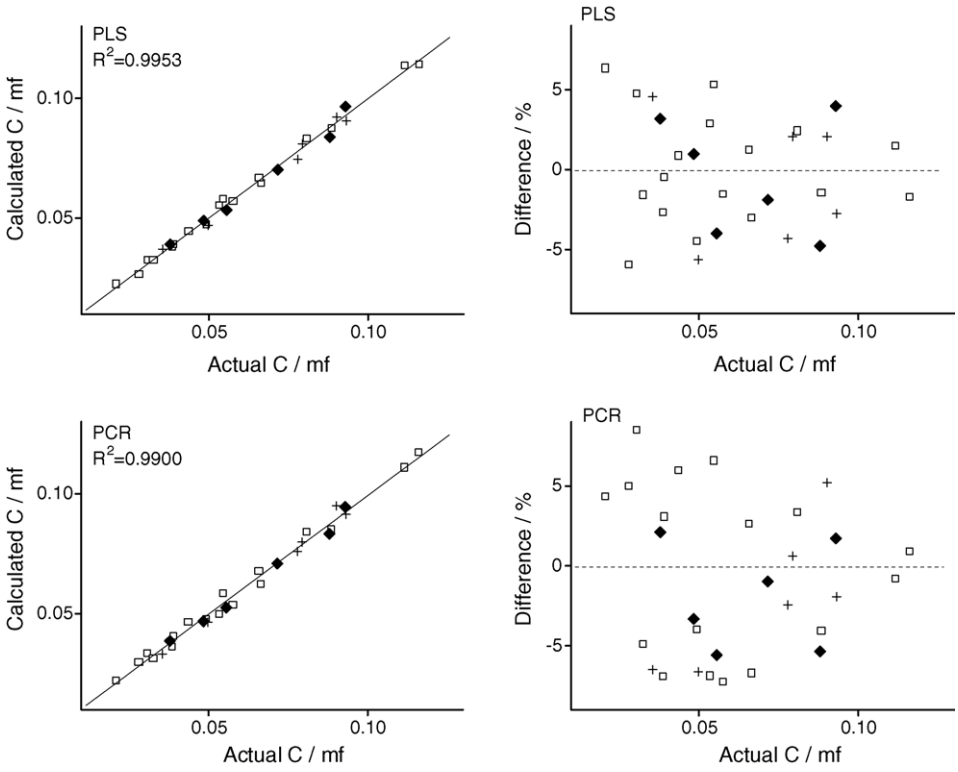

Fig. 4. Calibration curves and relative errors for prednisolone content obtained for PLS and PCR models based on unnormalised spectra; open symbols: calibration, plus: validation and filled symbols: testing data sets.

visible that errors found for PLS model are of the same order as those for captopril system. Mean amount of prednisolone in the studied preparation was found to be 5.02 ± 0.04 and 4.87 ± 0.05 mg per tablet ($n = 5$) from the PLS and PCR models, respectively.

There is gelatine amongst constituents of the studied system. This substance is a weak Raman scatterer. One of its strongest and widest bands overlaps with the main prednisolone line (Fig. 3). Quantitative analysis based on the calibration system in which gelatine was absent resulted in a systematic error. Prednisolone content determined from this model was approximately 20% higher than the correct one in the studied tablets.

3.4. Prednisolone tablets, internal standard added

In the next stage an appropriate amount of potassium ferrocyanide as an internal standard was added to each mixture. Spectra were recorded once more and normalised by the intensity at maximum or the integrated intensity of the ν_s(CN) band, and new models were constructed. The RSEP values found were comparable with those calculated for models based on undivided spectra. For the PLS models they do not exceed 2.2% for calibration set and 3.8% for testing data set in the case of prednisolone quantification, Table 3.

It should be noticed that Raman intensity scattered by the examined samples is rather low. So, to obtain acceptable S/N ratio, it was necessary to accumulate more than 500 interferograms. The poor S/N ratio could noticeably influence the quality of quantification, especially for models based on normalised spectra [20].

Mean content of prednisolone in the studied preparation found from the PLS (PCR) models ($n = 5$) equals 4.95 ± 0.19 mg (5.07 ± 0.15 mg) from spectra normalised by the intensity at maximum and 4.92 ± 0.16 mg (4.94 ± 0.18 mg) from spectra normalised by the integrated intensity of internal standard line. They are in a good agreement with the value obtained using UV–vis pharmacopoeial method [15], which gave 4.97 ± 0.05 mg of prednisolone per tablet ($n = 5$).

4. Conclusions

This study confirms the high potential of FT-Raman spectroscopy combined with multivariate calibration in the quantitative analysis of tablets with relatively low API content from a few to ca. 17%. Five commercial preparations were successfully quantified on the basis of different multivariate models. Found actives concentrations agree with those obtained using pharmacopoeial methods.

The applied procedure gives for solid mixtures a level of accuracy comparable with that characteristic for other analytical methods. Additionally, it does not require any special preparation of the tablets to be analysed. The proposed method is simple and it could have potential applications in industry as the analytical procedure for fast captopril, prednisolone and similar APIs quantification in tablets.

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