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Short communication

Quantification of atorvastatin calcium in tablets by FT-Raman spectroscopy

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ABSTRACT

The FT-Raman quantification of atorvastatin calcium in tablets was performed using the partial least squares (PLS), principal component regression (PCR) and counter-propagation artificial neural networks (CP-ANN) methods. To compare the predictive abilities of the elaborated models, the relative standard errors of prediction (RSEP) were calculated. The application of PLS, PCR and 6 × 6 CP-ANN provided models of comparable quality. RSEP error values in the range of 1.9–2.8% for calibration and validation data sets were obtained for the three procedures applied. Four commercial products containing 10, 20 or 40 mg of atorvastatin calcium per tablet were successfully quantified. Concentrations found from the Raman data analysis correlate strongly with the declared values, with a recovery of 98.5–101.3%, and with the results of reference analysis, with the recovery of 98.9–102.1%, for the different models. The proposed procedure can be a fast, precise and convenient method of atorvastatin calcium quantification in commercial tablets.

1. Introduction

Atorvastatin calcium (ATC), [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt trihydrate, is a synthetic lipid-lowering agent, an inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase [1].

Chromatographic techniques such as HPLC [2–6] and LC–MS [7,8] are the most popular analytical methods for this active pharmaceutical ingredient (API) quantification. Other analytical techniques used for ATC quantification include electrophoresis [9] and UV–vis spectrometry [10–12]. The suitability of Raman spectroscopy to the quantitative analysis of ATC polymorphs was also demonstrated [13].

It is well established that Raman spectroscopy is an effective analytical method for quantification of complex mixtures, including pharmaceutical preparations [14,15]. The most important advantage of this technique is probably its simplicity, but the short analysis time and capacity for automation of the analytical procedure are also very important. With this method, there is no need to solvate or extract the API under consideration. In many cases, Raman spectroscopy, supported by chemometrics, enables the analysis of medicines in their unaltered states, without any sample treatment. It is a particularly useful tool in the analysis of products with a high API content [16,17]. Nevertheless, Raman quantification of preparations containing less than 5% by weight of API was also reported [18,19].

ATC is usually administered in the form of tablets containing from 10 to 40 mg of the active compound per tablet. This corresponds to ATC comprising not more than a few percent of the tablet mass. The difficulty of ATC quantification by Raman is compounded by its big formula weight, equal to 1155 Da. It is 6–8 times larger than the formula weights of acetylsalicylic acid and acetaminophen, active ingredients that are known to be easily quantifiable in solid dosage forms by this method [17]. It should be pointed out that the Raman technique is not a very sensitive one and the facts mentioned above may hamper reliable ATC quantification.

The advantage of neural networks over other chemometrics methods such as partial least squares (PLS) and principal component regression (PCR) [20,21] in the modeling of systems for which non-linear signal-answer dependencies are present is well documented [22]. Herein, we present the results of quantification by PLS, PCR and counter-propagation neural networks (CP-ANN) [23,24] data treatment of four commercial atorvastatin calcium tablets containing approximately 5–7% of active compound. The results obtained by the Raman technique are compared with those found from UV–vis measurements.

2. Experimental

2.1. Materials and sample preparation

The substances used, namely atorvastatin calcium and lactose, were of pharmacopoeial purity. The active component was kindly donated by Biofarm. Microcrystalline cellulose, hydroxypropyl

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methylcellulose, magnesium stearate, $CaCO_3$, and TiO_2 were of analytical grade (Sigma). Four atorvastatin calcium preparations, in the form of tablets containing, as declared, 10–40 mg of API, were purchased in a local pharmacy. Methanol used for UV–vis measurements was of HPLC grade (Merck).

Calibration and validation samples were prepared by mixing pure, solid substances in a mortar for several minutes to homogenize the powders properly. Suitable weight ratios of compounds were taken to minimize the colinearity between component concentrations and no significant correlations were observed. The determination coefficients R^2 for the concentration versus concentration plots were in the 0.01–0.16 range. Next, half of the prepared mixture, i.e., approximately 200 mg of powder, was used to prepare a pellet in a way similar to that adopted in IR spectroscopy. The commercial tablets were first ground and then processed further in the same way as the calibration samples.

Reference quantification of atorvastatin calcium preparations was performed according to an adopted spectrophotometric procedure given by Nagaraj et al. [11] and Sonavane et al. [12].

2.2. Apparatus

A Nicolet Magna 860 FT-IR spectrometer interfaced with an FT-Raman accessory with a CaF_2 beamsplitter and indium–gallium arsenide (InGaAs) detector was used to perform the measurements. The samples placed in a rotating sample holder were illuminated by an Nd:YVO_4 laser line at 1.064 μm with a power of 350 mW at the sample, without a converging lens; backscattered radiation was collected. Samples were rotated at a constant speed of ca. 200 rpm. The interferograms were averaged over 256 scans, Happ-Genzel apodized and Fourier transformed using a zero filling factor of 2 to produce spectra in the 100–3700 cm^{-1} range at a resolution of 8 cm^{-1}.

UV–vis spectra of ATC methanol solutions, in the range 200–300 nm with the resolution of 1 nm were recorded using a Carry-5 Varian spectrometer.

2.3. Software and numerical data treatment

Nicolet TQ Analyst ver. 7 chemometrics software was used to construct PLS and PCR models. The neural network simulations were performed with the help of software developed by Zupan [25]. The numerical data were prepared and transformed into an appropriate format using the Matlab (MathWorks) environment. All spectral data were mean centered.

To characterize and compare the predictive abilities of the developed models, the relative standard errors of prediction (RSEP) were calculated according to the following formula [26]:

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

in which C^A is the actual component content, C is the concentration found from Raman or UV–vis data analysis, and n is the number of samples. The $RSEP_{cal}$ and $RSEP_{val}$ errors were determined for the calibration and validation data sets respectively.

The cross-validation, using leave-one-out technique, was performed to estimate the robustness of the constructed models. The root mean square error of cross validation (RMSECV) was calculated to select an optimal number of factors for the PLS models.

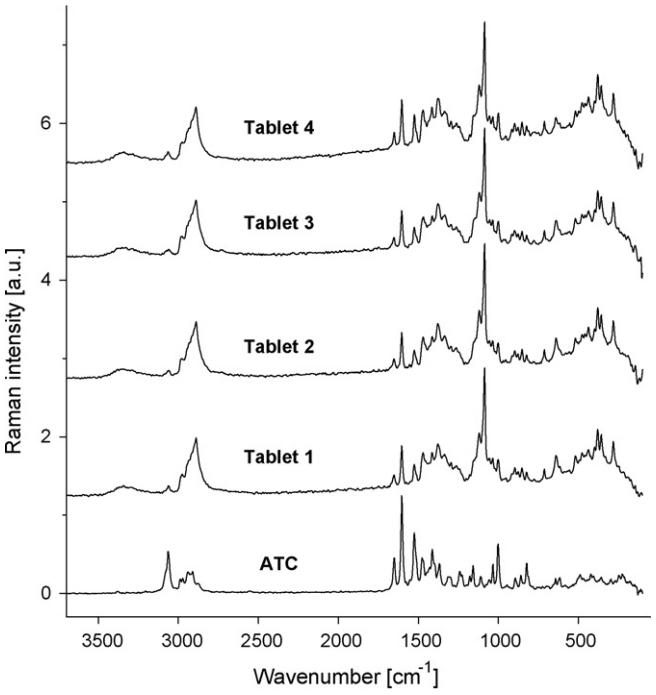

Fig. 1. FT-Raman spectra of atorvastatin calcium and four analyzed tablets.

3. Results and discussion

3.1. Raman analysis

In Fig. 1, the FT-Raman spectra of pure atorvastatin calcium and of the studied commercial preparations are presented. All four analyzed tablets, denoted 1, 2, 3 and 4, besides the active component (5.0–7.0% by weight), contain as additives lactose, cellulose and its hydroxypropyl methyl derivative, magnesium stearate, $CaCO_3$, and TiO_2 in different proportions. Tablets 1, 2 and 3 were produced by the same manufacturer, and the qualitative composition of the tablet mass of these preparations was very similar. The differences between the content of the studied commercial samples originating from the two manufacturers can be attributed mainly to the different composition of the outer layer of the tablets. Although compounds present in tablet coatings are rather weak Raman scatterers, the differences in tablet composition are clearly shown by the scores plots of PCA analysis.

To construct calibration models, the Raman spectra of 36 solid samples (prepared as described above) were used. The mass fraction varied in the 0.03–0.10 range for atorvastatin calcium, 0.17–0.39 for lactose, 0.31–0.55 for cellulose, 0.03–0.19 for hydroxyproplyl methylcellulose, 0.005–0.05 for magnesium stearate, 0.09–0.17 for $CaCO_3$, and 0.005–0.03 for TiO_2. Eight mixtures were chosen for the validation procedure, and the validation data set was selected by the Kohonen mapping. The remaining 28 samples were used as a calibration or training set. This division between calibration and validation samples was preserved for all PLS, PCR and CP-ANN models built. In the construction of the chemometrics model, the 990–1680 cm^{-1} region of Raman spectra was applied. Usually this region was slightly modified for each preparation studied. From the RMSECV plot for ATC presented in Fig. 2 (top left), it follows that it is enough to build the PLS model with the use of 4 factors.

In comparison to the PLS and PCR methods, the optimization of neural networking appears to be more complex because the number of possible network parameters influencing the strength of the

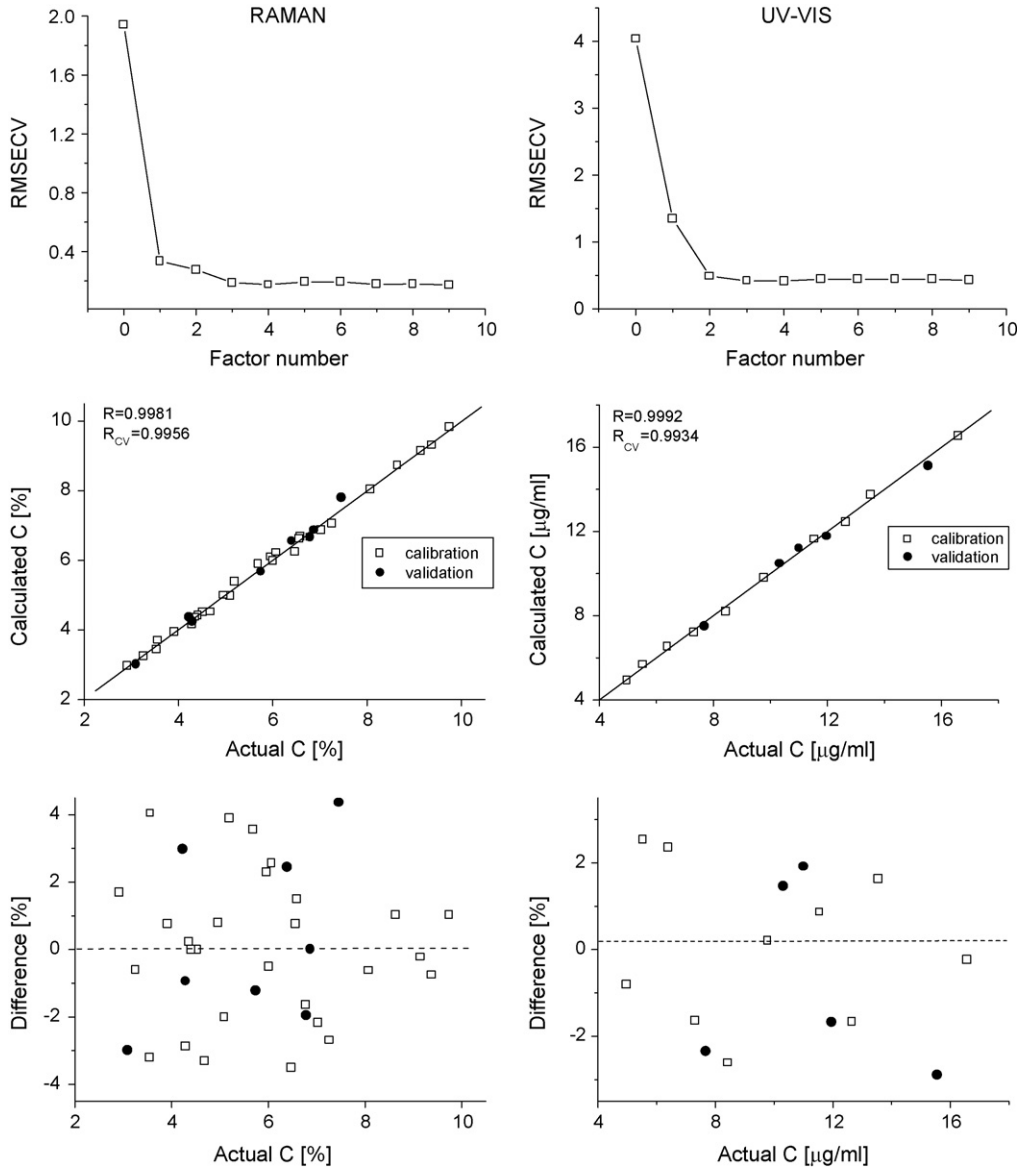

Fig. 2. RMSECV values calculated for ATC modeling (top) based on Raman (left) and UV–vis (right) spectra. Calibration curves (middle) and relative errors (bottom) for ATC content obtained using the PLS model based on Raman (left) and UV–vis (right) spectra.

model is usually larger. The settings of the CP-ANN parameters were adjusted by treating the set of calibration samples represented by Raman intensities at selected wavenumbers, ca. 700 points, as inputs. During the screening procedure, the number of neurons in the *x*- and *y*-direction N_x (=N_y) from 4 to 7 was checked, and the number of learning epochs in the 50–500 range was screened. The networks were trained using standard values of the maximal and the minimal correction factor settings, i.e., 0.5 and 0.01.

The upper left panel in Fig. 3 shows the results of the screening procedure for the modeling of tablet 1. Improvement of the $RSEP_{cal}$ parameter values is observed with the increase of network dimension and with the elongation of the learning process. Relative standard errors of prediction found for the calibration set do not exceed 2% in the case of $N_x = 6$ and 7 networks, while for the smallest CP-ANN ($N_x = 4$), their values are 2 times higher.

The plot of RSEP error values for validation samples presented in the upper right panel of Fig. 3 is more complex in comparison with the analogous plot for the training set. The low $RSEP_{val}$ errors occur for particular arrangements of the CP-ANN only, and in the case of the studied tablet, the lowest error was obtained for a 6 × 6 network trained by 100 learning cycles. Also, for other preparations studied, the 6 × 6 networks were among those giving the best predictions for the validation set.

The RSEP error values found for the calibration and validation sets for the three procedures applied are quoted in Table 1. As one can see, there is no noteworthy difference in the quality of the models resulting from different computational approaches used. They manage to effectively model the concentration-dependent changes in spectral data. The $RSEP_{cal}$ changes from 1.9 to 2.7%, while $RSEP_{val}$

Table 1
Calibration parameters for atorvastatin calcium in tablets.

Method	*R*	$RSEP_{cal}$ (%)	$RSEP_{val}$ (%)	R_{cv}
PLS	0.998	1.89	2.66	0.995
PCR	0.996	2.66	2.60	0.990
CP-ANN	0.998	1.98	2.85	0.987
PLS UV–vis	0.999	1.41	2.26	0.994

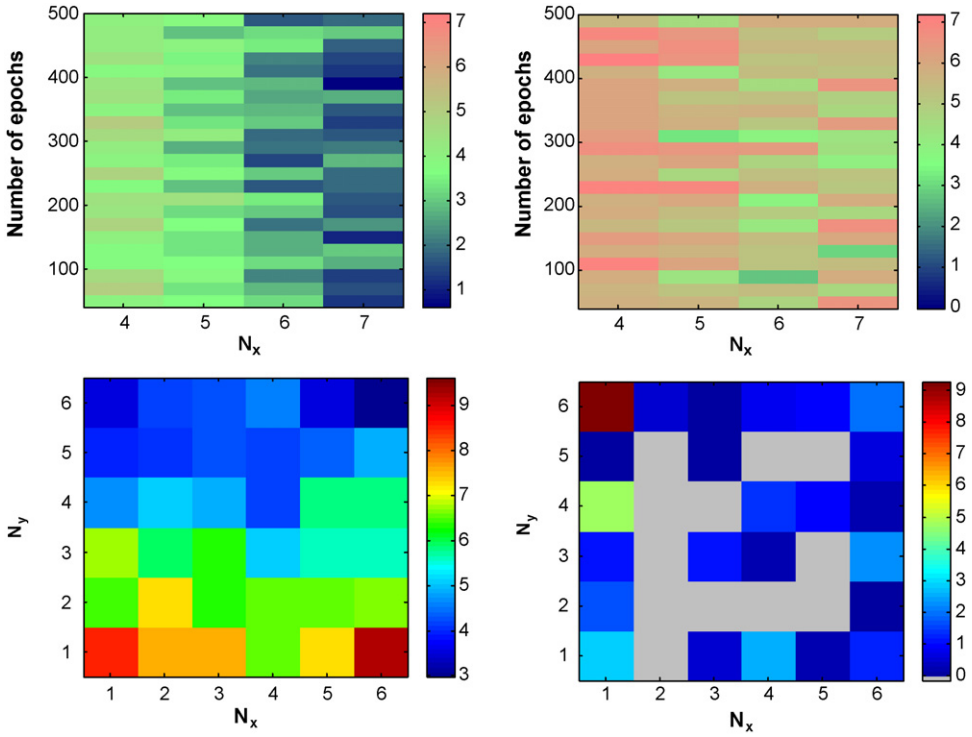

Fig. 3. CP-ANN networking: results of screening procedure for CP-ANN: $RSEP_{cal}$ and $RSEP_{val}$ errors (%) as functions of the number of neurons in the *x*- and *y*- directions and the number of learning cycles (upper panel). The top map of predicted atorvastatin calcium concentrations (left) [%] and the top map of the relative accuracy [%] for the training data set (right); grey indicates empty neurons (lower panel).

is found to be in the 2.6–2.8% range. All three models are characterized by comparable resistance to the leave-one-out internal validation procedure, producing comparable R_{cv} values (0.99). The typical calibration curve and plot of relative errors for the PLS model are shown in Fig. 2 (left middle and bottom). The top map of the predicted active compound concentrations and the top map of relative errors for the calibration set obtained on the basis of the selected neural network for the modeling of tablet 1 is presented in Fig. 3 (lower panels).

On the basis of the constructed models, the studied tablets were quantified. The mean content of atorvastatin calcium in the analyzed commercial preparations determined by FT-Raman analysis is collected in Table 2. The presented values show that each of the three computational approaches applied is comparably efficient in the API quantification. In the case of the neural network quantification, one should take note that the standard deviation (SD) value depends strongly on the number of neurons to which analyzed samples are assigned; therefore, SD equals zero for samples assigned to the same neuron.

Quantification of the analyzed preparations gave atorvastatin calcium content with the recovery, calculated against declared values, in the range of 99.8–101.2%, 99.4–101.3% and 98.5–100.9% for the PLS, PCR and CP-ANN methods respectively. The mean concentrations of API found from Raman spectra agree perfectly well with the results of the reference analysis quoted in Table 2, with the recovery in the range 99.5–101.8%, 99.8–102.1 and 98.9–101.9, for the three methods applied. This is shown in Fig. 4.

3.2. UV–vis analysis

Reference UV–vis analysis was performed according to a procedure described by Nagaraj and Sonavane et al. [11,12]. UV–vis spectra of ATC solutions in methanol containing 5.0–16.6 μg/ml of the active substance were recorded in the 200–300 nm range for 15 samples. Five mixtures were chosen for the validation procedure while the remaining 10 samples were used as a training set (Fig. 2). From the RMSECV plot for ATC presented in Fig. 2 (top right), it follows that it is enough to build the PLS model with the use of 3 factors. The parameters of the PLS model constructed are quoted in Table 1. On the basis of this model, the studied preparations were quantified. The mean content of ATC in the analyzed tablets is collected in Table 2.

Although this type of analysis is commonly regarded as cheap and simple, it is necessary to mention that we spent more than 10 l of methanol during UV–vis analysis of ATC for the substantially reduced number of samples.

Fig. 4. Recovery of ATC for the studied pharmaceuticals as percentages (%, w/w) of the declared amount (filled symbols) and of the amount found from reference analysis (open symbols) and different analytical models: PLS (squares), PCR (circles) and CP-ANN (triangles).

Table 2
Results (in milligrams) of FT-Raman analysis of the studied preparations ($n = 8$).

Method	Preparation (declared content)			
	Tablet 1 (10.34)	Tablet 2 (20.68)	Tablet 3 (41.36)	Tablet 4 (10.85)
PLS	10.32 ± 0.15	20.66 ± 0.58	41.28 ± 0.60	10.98 ± 0.33
PCR	10.47 ± 0.14	20.56 ± 0.78	41.64 ± 0.68	10.89 ± 0.33
CP-ANN	10.43 ± 0.74	20.38 ± 0.04	41.57 ± 0.92	10.79 ± 0.09
Reference analysis	10.37 ± 0.20	20.60 ± 1.07	40.80 ± 1.50	10.79 ± 0.45

4. Conclusions

This study confirms the high potential of FT-Raman spectroscopy combined with chemometric techniques in the quantitative analysis of pharmaceuticals with relatively low active ingredient content. Four commercial preparations of atorvastatin calcium in the form of tablets, containing 10, 20 or 40 mg of API per tablet (5–7% by weight) were successfully quantified using the PLS, PCR and CP-ANN models. Concentrations found from the Raman data analysis agree favorably with the results of reference analysis with the recovery in the 98.9–102.1% range. They also correlate strongly with the declared values, with a recovery in the 98.5–101.3% range for the different models elaborated. The proposed procedure can be a fast and accurate method of atorvastatin calcium quantification in commercial tablets, as well as a convenient way of simultaneous determination of other ingredients in the tablet mass.

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