Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 1235–1242
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Quantitative determination of diclofenac sodium and aminophylline in injection solutions by FT-Raman spectroscopy

Sylwester Mazurek, Roman Szostak *

Faculty of Chemistry, University of Wrocław, 14 F. Joliot-Curie, 50-383 Wrocław, Poland

Received 8 December 2004; received in revised form 2 September 2005; accepted 4 September 2005
Available online 8 November 2005

Abstract

The FT-Raman quantification of diclofenac sodium and aminophylline commercial injection solutions was performed. The efficiency of various spectra treatment procedures including classical univariate intensity ratio and multivariate partial least squares (PLS) and principal component regression (PCR) methods was compared. First, the calibration models were built using unnormalised spectra. Next, spectra normalised by the intensity of a selected band of CH_3CN added as an internal standard to the studied samples were utilised. To compare the predictive ability of the models constructed, the relative standard error of prediction (RSEP) was calculated. The errors found for multivariate calibrations were a few times smaller than those for the univariate ones. Usually, the most effective was the PLS method, for which RSEP values of the order of 1–2% for calibration and 2–3% for testing data sets were obtained.

Four commercial preparations of diclofenac sodium and one of aminophylline containing by weight, 2.4% of the active pharmaceutical ingredient (API) were quantified applying the developed models. Concentrations found from the Raman data analysis correlate with the declared values and the results of reference analyses. For the studied diclofenac sodium solutions they amount to 99.2–101.2% of the former and 101.2–102.4% of the latter quantities for the PLS models optimised for each medicine based on unnormalised spectra. These values for the aminophylline preparation were found to be 101.0 and 99.1%, respectively. It shows that the proposed procedure based on the chemometric treatment of FT-Raman spectra can be a fast and convenient alternative to the standard pharmacopoeial procedures of API quantification even in relatively diluted injection solutions.

Keywords: FT-Raman spectroscopy; Diclofenac sodium; Aminophylline; Theophylline; Injection solutions; Quantitative determination

1. Introduction

It is well-established that Raman spectroscopy is an effective analytical method in the quantification of complex mixtures, including pharmaceutical preparations [1–4]. Unfortunately, this technique is not commonly recognised as an alternative to pharmacopoeial procedures, although it enables analysis of medicines in the form of tablets, capsules and solutions, often without any additional sample treatment, which simplifies and shortens the analysis. It is a particularly useful tool in the analysis of tablets with a high active pharmaceutical ingredient (API) content [5–9]. Raman quantification of systems with <10% active component concentration, expressed in weight units, is not widespread [10]. Quantitative Raman studies of injection solutions are especially rare even though this method can be employed to assay solutions in their original glass or plastic ampoules.

There are different approaches to API quantification using the Raman technique. In the first, calibration mixtures and analysed preparations have the same composition. This approach is used during on-line process control in factories where all constituents of the analysed sample are known. Sometimes during analysis the detailed pharmaceutical composition is not known. In such a case it is possible to build a simplified calibration model based on samples containing only an active substance and the main diluent present in the studied preparations and to perform analysis using an internal or external standard method. Based on this two–three component system the quantitative analysis of various preparations for the same API is possible. However, during the construction of the model, it is necessary to avoid spectral ranges where unidentified compounds could interfere.

In the present work, results of FT-Raman quantification of commercial injection solutions containing ca. 2.4% of diclofenac sodium and ca. 2.4% of aminophylline (2.0% of theo-

* Corresponding author. Tel.: +48 71 3757238; fax: +48 71 3282348.
E-mail address: rsz@wchuwr.chem.uni.wroc.pl (R. Szostak).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.09.019

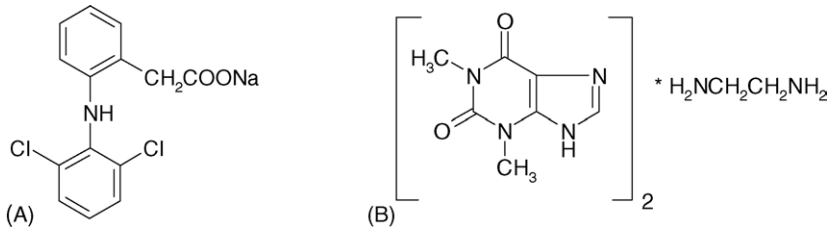

Fig. 1. Chemical structure of diclofenac sodium (A) and aminophylline (B).

phylline) are presented. Whereas the composition of diclofenac sodium preparations is well-defined, in the case of aminophylline preparations they may contain an excess of ethylenediamine [11].

During analysis, first univariate and multivariate models were built on the basis of unnormalised spectra. Next, an internal standard was added to samples and spectra normalised by their selected band intensity were used to construct calibration models.

Diclofenac sodium (Fig. 1A), a sodium salt of 2-[(2,6-dichlorophenyl)aminophenyl]-acetic acid is a potent analgesic and anti-inflammatory agent, commonly used in various drug formulations, including tablets, capsules, drops, injections, suppositories, ointments and gels [11]. Several analytical methods of diclofenac sodium quantification in pharmaceuticals have been developed. Among them the UV–vis spectrometry [12–16], HPLC [17,18], LC [19–21], densitometry [22] and potentiometry [23,24] or spectrofluorometry [25–27] can be listed as the most widespread. The possibility of Raman spectroscopy application to its quantitative analysis was also noticed [28].

Aminophylline (Fig. 1B) is a xanthine broncholidator. It is a complex of two theophylline molecules with a molecule of ethylenediamine containing not <84.0% and not >87.4% theophylline and the equivalent of 13.5–15.0% ethylenediamine, both calculated with reference to the anhydrous substances. Pharmacopoeial methods applied to its quantification are based on theophylline determination [11]. Other analytical techniques of aminophylline determination include HPLC [29] and chemiluminescence measurements [30].

2. Experimental

2.1. Materials and sample preparation

The substances used, namely diclofenac sodium, theophylline, ethylenediamine, benzyl alcohol, 1,2-propanediol, D-mannitol, NaOH and sodium pyrosulfite were of pharmacopoeial or analytical purity.

Aqueous solutions were prepared using purified water characterised by a resistivity >18 MΩ cm. Four preparations of diclofenac sodium D1–D4 and one of aminophylline (A3), containing a declared 25 mg/mL of API were purchased in a local pharmacy.

Samples with suitable compound weight ratios were prepared by mixing all constituents present in the studied preparations. To avoid the collinearity between concentrations of active components and remaining constituents of the studied solutions, concentration versus concentration graphs were plotted. No significant correlations were observed. The largest determination coefficient R^2 for these plots amounted to 0.106. In the case of diclofenac sodium mixtures, a solution of sodium hydroxide, D-mannitol and $Na_2S_2O_5$ in water was prepared first. The concentrations of these substances by weight, equalled 0.12, 0.58 and 0.45%, respectively. Next, the active ingredient, propylene glycol and benzyl alcohol were added to the prepared solution. To obtain the required concentrations of aminophylline, appropriate amounts of theophylline and ethylenediamine were dissolved in water.

In the second step, an approximately constant volume of acetonitrile, chosen as an internal standard was added to each sample.

2.2. Reference diclofenac sodium and aminophylline analysis

Reference quantification of diclofenac sodium preparations was performed according to an elegant recipe given by de Micalizzi et al. [15]. Seven solutions containing from 11.0 to 41.2 μg/mL of diclofenac sodium and 38.6 μg/mL of benzyl alcohol were prepared in water. Using the first derivative of UV–vis spectra, a calibration curve (slope = 4.67×10^{-4}, intercept = -3.0×10^{-5}, $R^2 = 0.9973$) was constructed by the zero-crossing technique (λ = 257.8 nm).

Reference analysis of theophylline content in aminophylline solution was carried out using UV–vis spectrometry according to the method described in the British Pharmacopoeia [11].

2.3. Apparatus

A Nicolet Magna 860 FT-IR spectrometer interfaced with a FT-Raman accessory equipped with CaF_2 beamsplitter and indium–gallium–arsenide (InGaAs) detector was used to carry out the measurements. The solutions placed in the same NMR tube were illuminated by a Nd:YVO_4 laser line at 1.064 μm with a power of ca. 570 mW at the sample without a converging lens and backscattered radiation was collected. The interferograms were averaged over 512 scans, Happ–Genzel-apodized and Fourier-transformed using a zero filling factor of 2 to give spectra in the 100–3700 cm^{-1} range at a resolution of 8 cm^{-1}. Under such conditions it took approximately 10 min to obtain the spectrum.

UV–vis spectra were recorded using a Carry-5 Varian spectrometer. The density of samples at 20 °C was measured using an Ecolab MG-2 densimeter.

2.4. Chemometric models

Nicolet TQ Analyst chemometric software was used to construct univariate and multivariate models and to perform the quantitative analysis of the commercial products. During the course of multivariate analyses, spectra were mean-centred. Generally the quantitative composition of the studied samples was expressed as wt%, except for models based on spectra normalised by the acetonitrile ν_s(CN) band intensity for which a weight ratio was used instead.

To characterise the prediction ability of developed calibration models and compare them the relative standard error of prediction, RSEP, was calculated according to the equation:

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

in which C^{A} is the actual component content, C the concentration found from Raman 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 partial least squares (PLS) models.

3. Results

3.1. Quantification of pure injection solutions

In Fig. 2 the FT-Raman spectra of the four analysed diclofenac sodium commercial solutions are shown. A qualitative analysis of the pharmaceuticals was performed first. All four investigated solutions, beside the active component, contain water, propylene glycol and benzyl alcohol as the main additives. Small amounts of sodium hydroxide, D-mannitol and sodium pyrosulfite or *N*-acetylcysteine were also detected. They increase the solubility

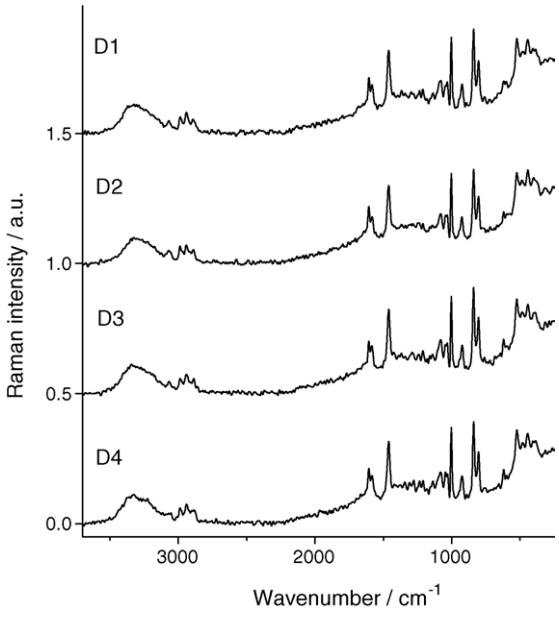

Fig. 2. FT-Raman spectra of the four analysed commercial diclofenac sodium injection solutions D1–D4; the spectra are offset for clarity by 0.5.

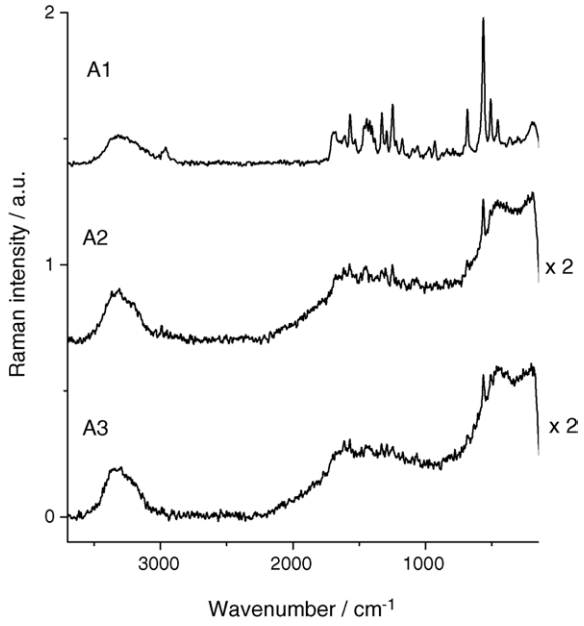

Fig. 3. FT-Raman spectra of aminophylline 25% solution (A1), calibration sample (A2) and aminophylline preparation (A3); the spectra are offset for clarity by 0.7.

of diclofenac in water or preserve solutions from oxidation. The measured density of the analysed products was found to be 1.0315, 1.0317, 1.0306 and 1.0321 g/mL for D1, D2, D3 and D4 preparations, respectively.

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 concentration varied in the range 1–4% for diclofenac sodium, 10–28% for propylenediol, 1–5% for benzyl alcohol and 64–83% for the aqueous solution of NaOH, D-mannitol and sodium pyrosulfite.

The second analysed system consists of three substances only. Aminophylline, which is a complex of theophylline with ethylenediamine (2:1) is dissolved in water. As mentioned before, it may contain an excess of ethylenediamine. In the spectra of diluted aminophylline solutions there are only two peaks at 564 and 683 cm^{-1}, clearly visible for the active compound (Fig. 3).

The 36 calibration samples were prepared by dissolving appropriate amount of theophylline in ethylenediamine water solution. The calibration set consisted of 24 mixtures, 6 mixtures were chosen for the validation procedure and 6 other were treated as testing samples. The concentration varied in the range 1–5% for theophylline, 1–7% and 89–98% for ethylenediamine and water, respectively. Raman spectra of these mixtures were recorded under the same conditions as for diclofenac sodium solutions.

Because of the low active component concentration in the studied pharmaceuticals, the intensities of the measured spectra were weak. It means that the spectra obtained were characterised by a rather low signal-to-noise (S/N) value of the order of 30. In the case of diclofenac sodium solutions the principal component analysis (PCA) showed that in such a complex system only about

Table 1
Calibration parameters for API in diclofenac sodium solutions

Normalisation	Parameter	Calibration model			
		PLS	PCR	Univariate method	
				Band area	Band intensity
Unnormalised	RSEC[a] (%)	2.95	3.09	11.2	13.0
	RSEV[b] (%)	2.06	2.47	8.2	9.4
	RSEP (%)	2.21	2.65	11.5	13.7
	R^2	0.9953	0.9921	0.8522	0.7957
	Line equation			$I = 0.8381c + 0.0057$	$I = 0.7804c + 0.0073$
By intensity at maximum[c]	RSEC (%)	2.37	2.32	11.3	12.8
	RSEV (%)	2.29	2.57	10.4	12.0
	RSEP (%)	2.25	2.20	11.9	12.1
	R^2	0.9946	0.9942	0.8379	0.7906
	Line equation			$I = 0.7499c + 0.0086$	$I = 0.7068c + 0.0102$
By integrated intensity[d]	RSEC (%)	2.18	2.38	11.2	12.9
	RSEV (%)	2.32	1.94	10.4	12.0
	RSEP (%)	2.13	1.94	11.3	11.7
	R^2	0.9937	0.9948	0.8390	0.7885
	Line equation			$I = 0.7580c + 0.0084$	$I = 0.7126c + 0.0100$

[a] Relative standard error of calibration.
[b] Relative standard error of validation.
Spectra normalised by ν_s(CN) acetonitrile band intensity.
[c] At maximum.
[d] Integrated.

85% of the spectral variation could be accounted for by the first five principal components. The next principal components connected with the noise and fluctuations of the baseline decreased slowly. After smoothing of the spectra, the first five principal components accounted for nearly 94% of the spectral variation in the studied system, however smoothing had negligible influence on the parameters of the elaborated calibration models.

At the beginning, isolated diclofenac peaks at 1581 and 1604 cm^{-1} were chosen to perform analysis in a classical way, using a univariate approach. It was necessary to assume that the remaining substances, present in the studied systems, did not interfere strongly in this spectral region. Band intensities and band areas were calculated applying one-point baseline correction. As one could expect, univariate calibration models were of

Table 2
Calibration parameters for API in aminophylline solutions

Normalisation	Parameter	Calibration model			
		PLS	PCR	Univariate method	
				Band area	Band intensity
Unnormalised	RSEC[a] (%)	0.86	4.34	7.61	8.11
	RSEV[b] (%)	1.82	2.35	4.27	5.97
	RSEP (%)	2.69	2.34	8.93	8.89
	R^2	0.9988	0.9830	0.9487	0.9386
	Line equation			$I = 0.9228c + 0.0026$	$I = 0.9208c + 0.0027$
By intensity at maximum[c]	RSEC (%)	1.13	4.46	5.56	5.37
	RSEV (%)	3.30	2.41	3.85	4.24
	RSEP (%)	2.91	2.30	5.43	6.47
	R^2	0.9970	0.9822	0.9714	0.9720
	Line equation			$I = 0.9440c + .0077$	$I = 0.9416c + 0.0075$
By integrated intensity[d]	RSEC (%)	1.51	4.58	6.28	5.53
	RSEV (%)	2.26	2.29	4.62	5.40
	RSEP (%)	2.81	1.91	6.36	6.45
	R^2	0.9976	0.9804	0.9620	0.9681
	Line equation			$I = 0.9374c + 0.0063$	$I = 0.9493c + 0.0048$

[a] Relative standard error of calibration.
[b] Relative standard error of validation.
Spectra normalised by ν_s(CN) acetonitrile band intensity.
[c] At maximum.
[d] Integrated.

low quality. The determination coefficient, R^2, for obtained calibration curves equalled 0.852 or 0.796 (Table 1). The relative standard error of prediction determined for the testing data set amounted to 11.5 and 13.7%, for the model based on band area and band intensity, respectively.

In an attempt to build univariate calibration models for aminophylline solutions, the region in the vicinity of the 564 cm^{-1} theophylline line was used. Although the regression curves obtained were characterised by higher R^2 values than for diclofenac sodium solutions: 0.949 based on band area and 0.939 based on band intensity, the RSEP errors of 8.9% for both models were unacceptably high (Table 2).

To improve the quality of the analysis, two multivariate methods, namely PLS and principal component regression (PCR) were applied. The following spectral ranges 2897–2864, 1625–1558, 1497–741 and 564–422 cm^{-1} were chosen for diclofenac sodium solutions. Results obtained were evidently better than those for univariate models. The calibration curves for API and additives were characterised by R^2 values in the range of 0.992–0.997. This is depicted in Fig. 4 for API. In Table 1 the RSEP values found for the calibration, validation and testing samples using PLS and PCR models are quoted.

The model in which a partial least squares regression algorithm was used worked slightly better than the one based on principal component regression. The errors found for diclofenac sodium determination in testing samples equal 2.2 and 2.6%, respectively. RSEP values for additives varied in the 0.4–2.4% range for the PLS model and in the 0.5–2.1% range for the PCR one.

In the course of constructing multivariate models for aminophylline, two spectral ranges were applied: 3570–2587 and 1716–415 cm^{-1}. The calibration curves for theophylline were characterised by R^2 values in the range 0.983–0.999 (Fig. 5). The RSEP values obtained for theophylline in the case of the testing sample set equal 2.7% for PLS and 2.3% for PCR methods. A full set of calibration results for the API is collected in

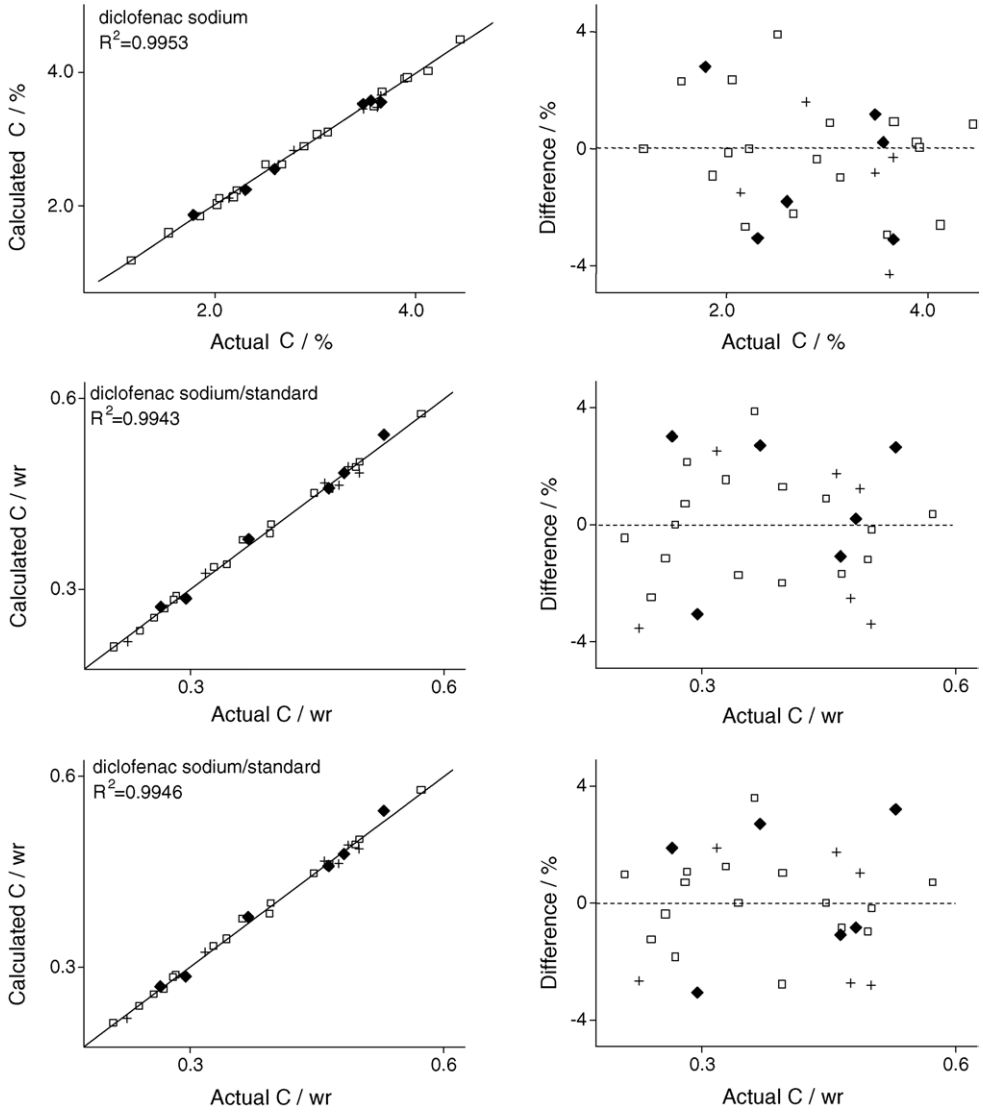

Fig. 4. Calibration curves and relative errors calculated for diclofenac sodium determination based on PLS models using unnormalised spectra (top) and normalised by the intensity at maximum (middle) and integrated intensity (bottom) of the ν_s(CN) acetonitrile band; open symbols, calibration; plus, validation and filled symbols, testing data sets.

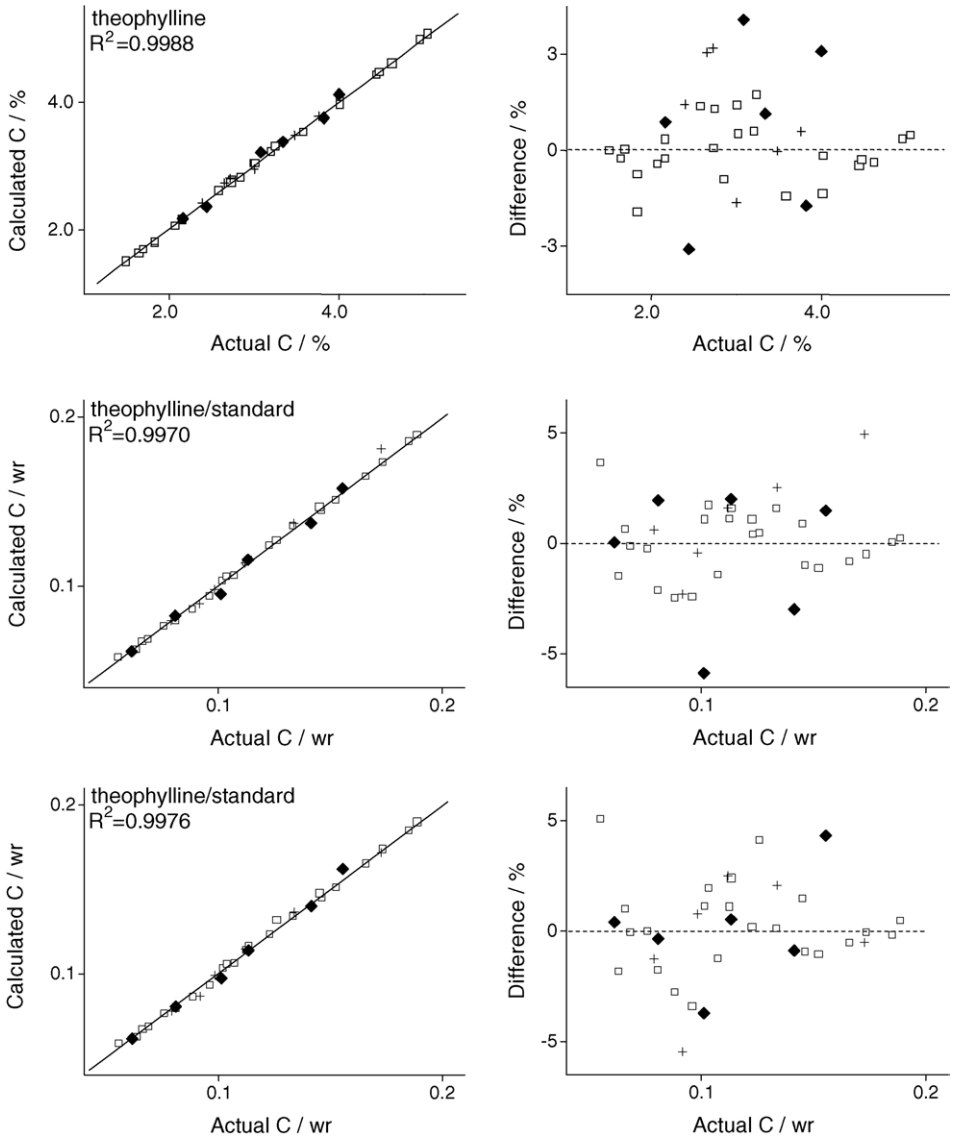

Fig. 5. Calibration curves and relative errors calculated for theophylline determination in aminophylline solutions based on PLS models using unnormalised spectra (top) and normalised by the intensity at maximum (middle) and integrated intensity (bottom) of the ν_s(CN) acetonitrile band; open symbols, calibration; plus, validation and filled symbols, testing data sets.

Table 2. As one could notice, the RSEP values for the multivariate models were even a few times smaller than those found for the univariate approach for both the studied APIs.

Applying calibration models described above commercial injection solutions were quantified. The amount of diclofenac sodium determined by FT-Raman method, from five independent analyses for each preparation is quoted in Table 3. Mean API concentration found in the studied medicines was in the range 24.4–25.6 mg/mL (24.8–25.3 mg/mL) based on the PLS model and 23.8–25.5 mg/mL (24.9–25.3 mg/mL) for the PCR one. In parentheses results obtained from models optimised for each studied solution, with spectral ranges slightly modified are quoted. As can be seen for optimised models the concentration ranges are narrower and RMSDs are smaller than those calculated for the uniform model. The concentrations obtained correlate well with the results of reference diclofenac sodium analysis [15] which gave 24.9 ± 0.8, 24.3 ± 0.5, 24.7 ± 0.7 and 24.9 ± 0.4 for the studied medicines D1, D2, D3 and D4, respectively. As can be easily checked there is no difference in mean concentrations found applying reference and both PLS procedures described above. At the 5% significance level, for a one-sided test and 9 d.f., the t-test values are always higher than $-t_{crit} = -1.833$ [31].

In Table 4, the quantification results for the commercial aminophylline solution are presented. The mean amount of theophylline in the analysed preparation, 21.2 mg/mL of solution, found on the basis of unnormalised spectra analysis from both PLS and PCR models correlates strongly with the theophylline content 21.0 ± 0.6 mg/mL obtained using the pharmacopoeial method ($n = 6$). For a one-sided test and 9 d.f., the t-test values are again higher than $-t_{crit}$, at the 5% significance level, for mean concentrations determined [31]. Lower concentrations of the theophylline were derived from univariate models, with noticeably higher standard deviation values.

Table 3
Results of diclofenac sodium analysis in commercial solutions (mg/mL)

Normalisation	Analysed preparation	Calibration model			
		PLS	PCR	Univariate method	
				Band area	Band intensity
Unnormalised	D1	25.1 ± 0.9 (25.2 ± 0.5)[a]	25.2 ± 1.1 (25.1 ± 0.6)	27.3 ± 1.2	26.8 ± 2.1
	D2	24.4 ± 0.5 (24.8 ± 0.4)	25.5 ± 0.9 (24.9 ± 0.4)	26.8 ± 0.8	25.3 ± 1.4
	D3	25.0 ± 0.9 (25.3 ± 0.5)	23.8 ± 1.1 (25.3 ± 0.8)	29.6 ± 3.5	28.3 ± 3.2
	D4	25.6 ± 0.9 (25.2 ± 0.3)	24.8 ± 0.6 (25.0 ± 0.5)	27.6 ± 3.3	27.2 ± 4.3
By intensity at maximum[b]	D1	25.8 ± 0.8 (25.1 ± 0.6)	25.6 ± 0.7 (24.9 ± 0.4)	29.8 ± 3.0	29.8 ± 3.2
	D2	24.7 ± 1.0 (25.2 ± 0.6)	24.6 ± 0.9 (25.5 ± 0.5)	24.8 ± 2.9	23.0±3.6
	D3	24.9 ± 1.1 (24.7 ± 0.6)	25.4 ± 0.9 (25.0 ± 0.5)	25.7 ± 3.3	25.9 ± 3.0
	D4	25.6 ± 0.5 (25.3 ± 0.4)	25.5 ± 0.7 (25.1 ± 0.6)	27.5 ± 2.0	27.1 ± 1.0
By integrated intensity[c]	D1	25.7 ± 1.0 (24.8 ± 0.4)	25.7 ± 0.6 (24.6 ± 0.6)	29.9 ± 3.1	29.8 ± 3.3
	D2	24.5 ± 1.5 (25.1 ± 0.3)	24.5 ± 1.3 (25.0 ± 0.4)	24.5 ± 3.2	24.2 ± 3.5
	D3	24.8 ± 1.0 (25.0 ± 0.8)	25.3 ± 1.0 (25.3 ± 0.6)	25.6 ± 3.3	25.8 ± 2.9
	D4	25.5 ± 0.9 (25.2 ± 0.6)	25.5 ± 1.1 (25.0 ± 0.6)	27.5 ± 2.0	27.1 ± 1.2

[a] In parentheses results obtained from optimised models.
Spectra normalised by ν_s(CN) acetonitrile band intensity.
[b] At maximum.
[c] Integrated.

Additionally, the main additives, namely propylene glycol and benzyl alcohol were quantified in the studied medicines based on the same calibration models. Their content is known for the preparations D2 and D4. Determined from the PLS approach, 196.3 and 189.5 mg of propanediol and 39.4 and 38.2 mg of alcohol in 1 mL of solution are close to the declared concentrations of 200 and 194 mg/mL of glycol and 40 mg/mL of benzyl alcohol, respectively. Concentrations of diclofenac sodium obtained from univariate models were noticeably higher than declared, which means that interference from other constituents of the studied mixtures cannot be neglected in the selected spectral region. Also, standard deviations found were considerably higher than those for the multivariate approach.

Table 4
Results of theophylline analysis in aminophylline commercial solutions (mg/mL)

Normalisation	Calibration model			
	PLS	PCR	Univariate method	
			Band area	Band intensity
Unnormalised	21.2 ± 0.5	21.2 ± 0.8	20.8 ± 3.0	19.9 ± 3.5
By intensity at maximum[a]	21.3 ± 1.0	21.9 ± 1.4	23.0 ± 0.4	22.7 ± 1.8
By integrated intensity[b]	21.3 ± 0.8	22.2 ± 0.8	22.7 ± 1.0	22.5 ± 2.2

Spectra normalised by ν_s(CN) acetonitrile band intensity.
[a] At maximum.
[b] Integrated.

3.2. Quantification of samples with internal standard added

Although the main constituents of the studied solutions are the same, there may be some substances added in small proportions, which can differ. In an attempt to improve the quantification of the studied medicines, acetonitrile was added as an internal standard to the mixtures and Raman spectra were recorded again. New models were constructed on the basis of spectra normalised by the acetonitrile 2254 cm^{-1} band intensity at maximum, with the baseline corrected at about 2227 cm^{-1} and the integrated intensity of this band calculated in the 2271–2236 cm^{-1} range. The calibration curves and relative errors for diclofenac sodium determination, using the PLS

method are shown in Fig. 4. For "unknown" samples, the errors for the API quantification amount to 2.1–2.3% (1.9–2.2%) after normalisation. This is presented in detail in Table 1. In parentheses, results for the PCR method are quoted.

An appropriate amount of acetonitrile as an internal standard was also added to each aminophylline mixture. Spectra were recorded once more and normalised by the ν_s(CN) band intensity at maximum or its integrated intensity. New models were constructed. In Fig. 5 the calibration curves and relative errors for theophylline quantification obtained from the PLS method were presented. The RSEP values found were comparable with those determined for models based on unnormalised spectra. They were in the range of 1.9–2.9% for the testing data set in the case of theophylline quantification (Table 2). The RSEP errors obtained from the univariate approach were slightly smaller than those found for unnormalised spectra.

Next, the commercial solutions with internal standard added were quantified on the basis of the developed models. The mean content of diclofenac sodium found in the studied preparations from the PLS models was in the 24.7–25.8 mg/mL (24.7–25.3 mg/mL) range based on spectra normalised by the intensity at maximum and in the 24.5–25.7 mg/mL (24.8–25.2 mg/mL) range based on spectra normalised by the integrated intensity of the acetonitrile ν_s(CN) band. Applying PCR models, these values were found to be in the 24.6–25.6 mg/mL (24.9–25.5 mg/mL) and 24.5–25.7 mg/mL (24.6–25.3 mg/mL) range, respectively. As in the case of unnormalised spectra results obtained from calibration models optimised for the each medicine studied are quoted in parentheses. Although the calibration parameters for univariate models were unsatisfactory, concentrations of the API found applying them were not very different from the real values, but once more quantification errors were unacceptably high (Table 3).

Mean content of theophylline in the studied aminophylline solution found from the PLS model equals 21.3 (21.3) mg/mL and 21.9 (22.2) mg/mL from the PCR model. Results obtained from spectra normalised by integrated intensity of the internal standard line are shown in parentheses (Table 4). Again, API concentration found from the PLS models is very close to that obtained by applying the pharmacopoeial method.

While normalisation of spectra reduces the influence of unstable environmental and/or spectral conditions, it simultaneously may be a source of additional factors which can interfere with the variance of the studied system [32]. In the case of spectra registered with a poor S/N ratio, the contribution of these factors could influence the quality of quantification. To minimise this effect, it is necessary to improve the S/N ratio, which can be achieved for example by a longer accumulation time.

4. Conclusions

Five commercial preparations containing 25 mg/mL of the API, four of diclofenac sodium and one of aminophylline were successfully quantified using the PLS models based on FT-Raman spectra. The proposed method is simple and it could have potential applications for fast and reliable API quantification in injection solutions.

References

[1] T.H. King, C.K. Mann, T.J. Vickers, J. Pharm. Sci. 74 (1985) 443–447.
[2] C. Wang, T.J. Vickers, C.K. Mann, J. Pharm. Biomed. Anal. 16 (1997) 87–94.
[3] T.M. Niemczyk, M. Delgado-Lopez, F.S. Allen, J.T. Clay, D.L. Arneberg, Appl. Spectrosc. 52 (1998) 513–518.
[4] M.J. Pelletier, Appl. Spectrosc. 57 (2003) 20A–42A.
[5] S.G. Skoulika, C.A. Georgiou, Appl. Spectrosc. 55 (2001) 1259–1265.
[6] R. Szostak, S. Mazurek, Analyst 127 (2002) 144–148.
[7] S.G. Skoulika, C.A. Georgiou, Appl. Spectrosc. 57 (2003) 407–412.
[8] A.O. Izolani, M.T. de Moraes, C.A. Téllez S., J. Raman Spectrosc. 34 (2003) 837–843.
[9] R. Szostak, S. Mazurek, J. Mol. Struct. 704 (2004) 229–233.
[10] M. Dyrby, S.B. Engelsen, L. Nørgaard, M. Bruhn, L. Lundsberg-Nielsen, Appl. Spectrosc. 56 (2002) 579–585.
[11] British Pharmacopoeia, HM Stationery Office, London, 1999.
[12] J.C. Botello, G. Pérez-Caballero, Talanta 42 (1995) 105–108.
[13] T. Pérez-Ruiz, C. Martinez-Lozano, A. Sanz, M.T. San Miguel, J. Pharm. Biomed. Anal. 16 (1997) 249–254.
[14] M.S. Garcia, M.I. Albero, C. Sánches-Pedreño, J. Molina, J. Pharm. Biomed. Anal. 17 (1998) 267–273.
[15] Y.C. de Micalizzi, N.B. Pappano, N.B. Debattista, Talanta 47 (1998) 525–530.
[16] M.L.F. de Córdova, P.O. Barrales, A.M. Díaz, Anal. Chim. Acta 369 (1998) 263–268.
[17] L. González, G. Yuln, M.G. Volonté, J. Pharm. Biomed. Anal. 20 (1999) 487–492.
[18] J. Klimeš, J. Sochor, P. Doležal, J. Körner, Int. J. Pharm. 217 (2001) 153–160.
[19] S.T. Patil, M. Sundaresan, I.C. Bhoir, A.M. Bhagwat, Talanta 47 (1998) 3–10.
[20] R. Hájková, P. Solich, M. Pospišilová, J. Šicha, Anal. Chim. Acta 467 (2002) 91–96.
[21] M.E. Abdel-Hamid, L. Novotny, H. Hamza, J. Pharm. Biomed. Anal. 24 (2001) 587–594.
[22] J. Krzek, M. Starek, J. Pharm. Biomed. Anal. 28 (2002) 227–234.
[23] O. Çakirer, E. Kiliç, O. Atakol, A. Kenar, J. Pharm. Biomed. Anal. 20 (1999) 19–26.
[24] A.M. Pimenta, A.N. Araújo, M.C.B.S.M. Montenegro, Anal. Chim. Acta 470 (2002) 185–194.
[25] P.C. Damiani, M. Bearzotti, M.A. Cabezón, A.C. Olivieri, J. Pharm. Biomed. Anal. 20 (1999) 587–590.
[26] L.A. Carreira, M. Rizk, Y. El-Shabrawy, N.A. Zakhari, S.S. Toubar, J. Pharm. Biomed. Anal. 13 (1995) 1331–1337.
[27] J.A. Arancibia, M.A. Boldrini, G.M. Escandar, Talanta 52 (2000) 261–268.
[28] M.C. Davies, J.S. Binns, C.D. Melia, P.J. Hendra, D. Bourgeois, S.P. Church, P.J. Stephenson, Int. J. Pharm. 66 (1990) 223–232.
[29] C. Yin, C. Tang, X. Wu, J. Pharm. Biomed. Anal. 33 (2003) 39–43.
[30] Z. Wang, Z. Zhang, Z. Fu, W. Luo, X. Zhang, Talanta 62 (2004) 611–617.
[31] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[32] R. Szostak, S. Mazurek, J. Mol. Struct. 704 (2004) 235–245.