Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Structure 704 (2004) 229–233
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

FT-Raman quantitative determination of ambroxol in tablets

Roman Szostak*, Sylwester Mazurek

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

Received 7 November 2003; revised 15 January 2004; accepted 15 January 2004

Available online 13 May 2004

Abstract

A procedure for quantitative determination of ambroxol in commercial tablets by Partial Least Squares (PLS) treatment of FT-Raman spectroscopic data is proposed. In a number of published reports, the quantification of pharmaceuticals, usually containing 30–90% of the analysed ingredient, with the application of the Raman technique is described. In the product under consideration, the active component constitutes less than 15% of the tablet mass.

A calibration model was built using unnormalized spectra and the results obtained were compared with values found from a second approach in which an internal standard was added to each sample. To appraise the quality of the PLS models, the relative standard error of prediction (RSEP) was calculated. For ambroxol, it amounts to 2.3–3.0% for a testing sample set quantification. The mean content of ambroxol in tablets determined from the model based on unnormalised spectra equals 29.6 ± 0.4 mg. It was found to be 29.6 ± 0.6 mg and 30.8 ± 0.3 mg from models built using spectra normalised by the intensity at maximum and integral intensity of the internal standard band, respectively. These values correlate well with the declared value of 30 mg of ambroxol in the studied medicine.

Keywords: FT-Raman spectroscopy; Ambroxol; Pharmaceuticals; Quantitative analysis

1. Introduction

There are several approaches to active ingredient quantification in tablets using the Raman technique. In the first approach, calibration mixtures contain only a studied agent and a tablet diluent, often lactose, starch or modified cellulose. Based on this two component system, the quantitative analysis of various preparations for the same active ingredient is performed [1,2]. As one could expect, the stated procedure can only work efficiently when these two substances constitute the prevailing majority of the tablet mass. Additionally, during the construction of the model, it is necessary to avoid spectral ranges where other substances could interfere. In the second, more natural, approach calibration models are built for each preparation separately [3]. It is rather a time consuming procedure, but it enables the accurate analysis of the active component content. To perform analysis of this type, it is necessary to find qualitative, or better, semi-quantitative composition of the studied preparation first, which sometimes may be a separate task. This method has potential applications in pharmaceutical and food industry. When not all constituents of the studied pharmaceutical are known, the addition of an internal standard could distinctly improve the quality of quantification [3,4].

Ambroxol hydrochloride is an active ingredient in a number of pharmaceutical preparations. This is a mucolytic agent that increases respiratory tract secretions, enhances pulmonary surfactant production and stimulates ciliary activity, which results in an improved mucus flow and transport. The enhancement of fluid secretions and muco-ciliary clearance facilitates expectoration and thereby eases coughing [5].

In the present work, the results of the quantification of commercial tablets containing ca. 14.9% of ambroxol are presented. Two procedures were applied. In the first one, a chemometric model was built on the basis of unnormalised spectra. In the second one, an internal standard was added to samples. Spectra were normalised by the selected internal standard band intensity and used to construct PLS models.

Lactose, $CaHPO_4$, $Ca_3(PO_4)_2$, magnesium stearate and sodium starch glycolate were found as additives in the studied preparation.

* Corresponding author.
E-mail address: rsz@wchuwr.chem.uni.wroc.pl (R. Szostak).

0022-2860/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2004.01.057

2. Experimental

A Nicolet Magna 860 FT-IR spectrometer interfaced with a FT-Raman accessory was used to carry out the measurements. The samples were illuminated by a Nd:YVO_4 laser line at 1.064 μm with a power of 0.42 W at the sample, without a converging lens. The interferograms were averaged over 1024 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}. In these conditions, it took approximately 20 min to obtain a spectrum.

In order to construct the calibration curves, samples with suitable compound weight ratios were prepared by mixing pure, solid substances in a mortar for a few minutes, to homogenise powders properly. Approximately 200 mg of powder were used to prepare a pellet. During the Raman spectra registration, samples were rotated at a speed of ca. 200 rpm. The commercial tablets were processed in a similar way. In the second step, an appropriate amount of potassium ferrocyanide, chosen as an internal standard, was added to each sample. New pellets were prepared as described above and Raman spectra were recorded again.

Nicolet TQ Analyst chemometric software was used to construct models and to perform the quantitative analysis of ingredients in the commercial product. All spectral data were normalised using a mean centering technique. The quantitative composition of the studied samples was expressed as a weight ratio for models constructed using spectra normalised by potassium ferrocyanide band intensity, or as a mass fraction for models based on undivided spectra.

In the present work, we report the results for a commercial medicine purchased in a local pharmacy. The substances used, namely ambroxol (2-amino-3,5-dibromo-*N*-[*trans*-4-hydroxycyclohexyl]benzylamine) hydrochloride, lactose and sodium starch glycolate were of pharmacopoeial purity. $CaHPO_4$, $Ca_3(PO_4)_2$, magnesium stearate and potassium ferrocyanide(II) trihydrate were of analytical grade.

3. Results and discussion

Ambroxol hydrochloride is a rather moderate Raman scatterer. Fig. 1 shows the FT-Raman spectra of pure ambroxol and the analysed commercial tablets. To construct the PLS model spectra of 28 samples prepared as mentioned above were used. Six mixtures were chosen for the validation procedure and six others were treated as the testing sample set. The mass fraction was varied in the 0.11–0.29 range for ambroxol, 0.24–0.50 for lactose, 0.02–0.18 for sodium starch glycolate, 0.10–0.32 for $CaHPO_4$, 0.04–0.12 for $Ca_3(PO_4)_2$ and 0.07–0.24 range for magnesium stearate. Lactose and sodium starch glycolate and both phosphates were treated as single constituents.

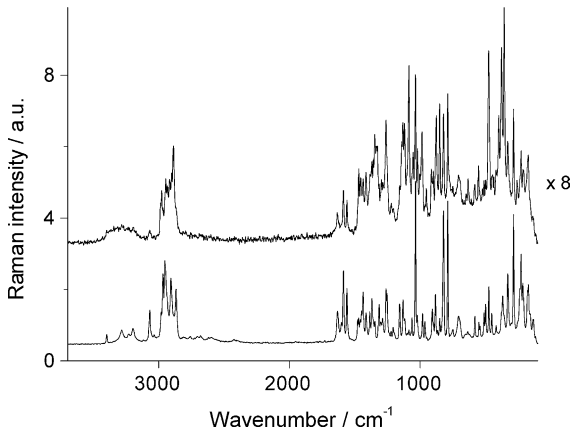

Fig. 1. FT-Raman spectra of analysed ambroxol tablets (top) and pure ambroxol (bottom).

The Raman spectra of these substances overlap strongly so it would be difficult to quantify them separately.

To characterise the prediction ability of the models, the RSEP was calculated according to the equation

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

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

The principal component analysis shows that in such a complex system, about 98% of the spectral variation could be accounted for by not less than five principal components. To obtain acceptable parameters of the constructed models, it was necessary to use at least 5–6 PLS factors. The following spectral ranges (baseline corrections in parentheses), were applied in the chemometric model construction: 3449–2833 (2771), 1726–1498 (1667), 1496–1233 (1185), 930–682 (929) and 430–193 (310) cm^{-1}. The calibration curves and relative differences for the studied samples are shown in Fig. 2, and the PRESS values for the analysed system, calculated according to Eq. (2), are presented in Fig. 3.

$$\mathrm{PRESS} = \sum_{i=1}^{n}(C_i - C_i^{\mathrm{A}})^2. \qquad (2)$$

In Table 1 the RSEP error values found for the calibration, validation and testing samples quantification are collected. It can be seen that for weak scattering constituents, such as calcium hydrogenphosphate, calcium phosphate or magnesium stearate, errors are two or even five times higher in comparison with the errors for ambroxol and lactose determination. The RSEP values found for ambroxol and lactose equal 2.3 and 1.9%, respectively. Based on the same calibration model, the studied pharmaceutical was quantified. The mean amount of an active ingredient found by

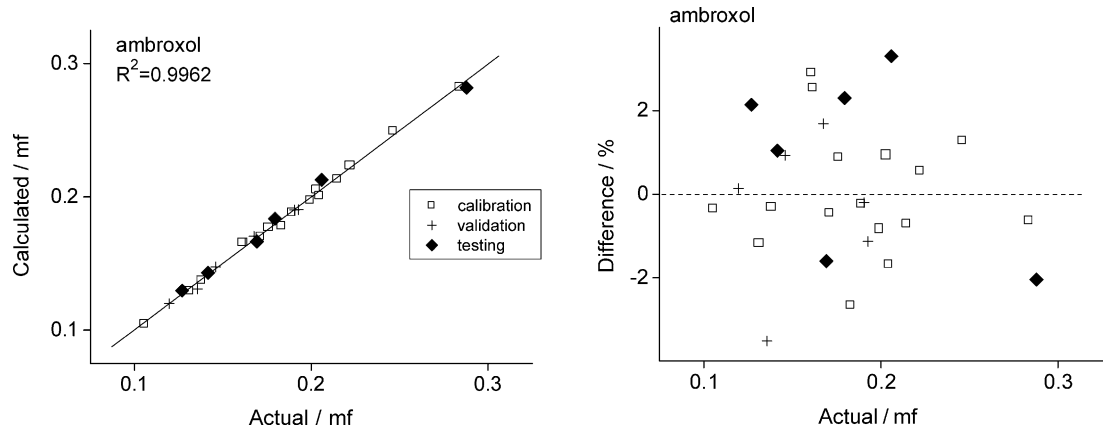

Fig. 2. Calibration curve and relative errors of ambroxol content; model constructed using unnormalised spectra.

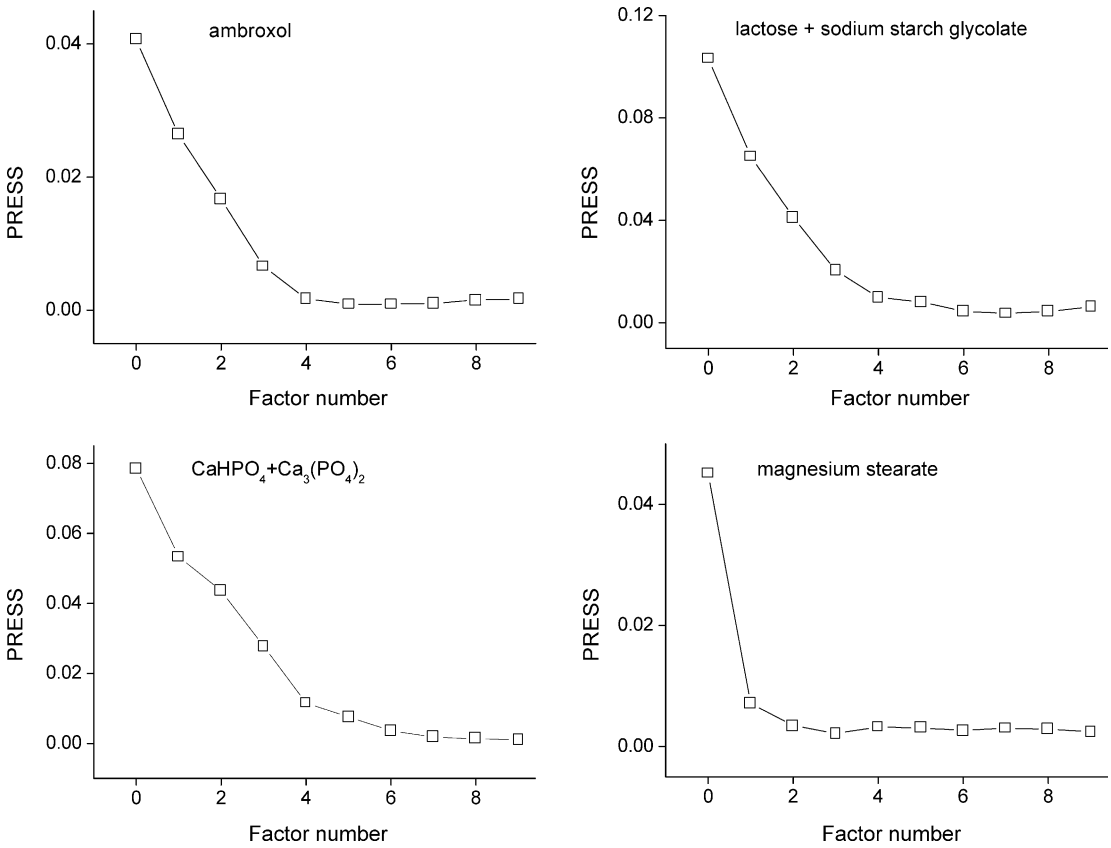

Fig. 3. PRESS values calculated for ambroxol tablet constituents based on unnormalised spectra.

Table 1
RSEP (%) values found from calibration models based on unnormalised spectra and spectra normalised by the 2091 cm^{-1} potassium ferrocyanide band

Samples	Unnormalised spectra				Normalised spectra	
	Ambroxol	Lactose + sodium starch glycolate	$CaHPO_4 + Ca_3(PO_4)_2$	Magnesium stearate	At maximum Ambroxol*	Integrated Ambroxol†
Calibration	1.33	1.76	2.29	4.44	1.82	1.99
Validation	1.56	2.87	3.25	10.26	2.79	3.71
Testing	2.27	1.87	3.43	12.74	3.00	2.95

* Spectra normalised by the intensity at maximum.
† Spectra normalised by the integrated intensity of the internal standard band.

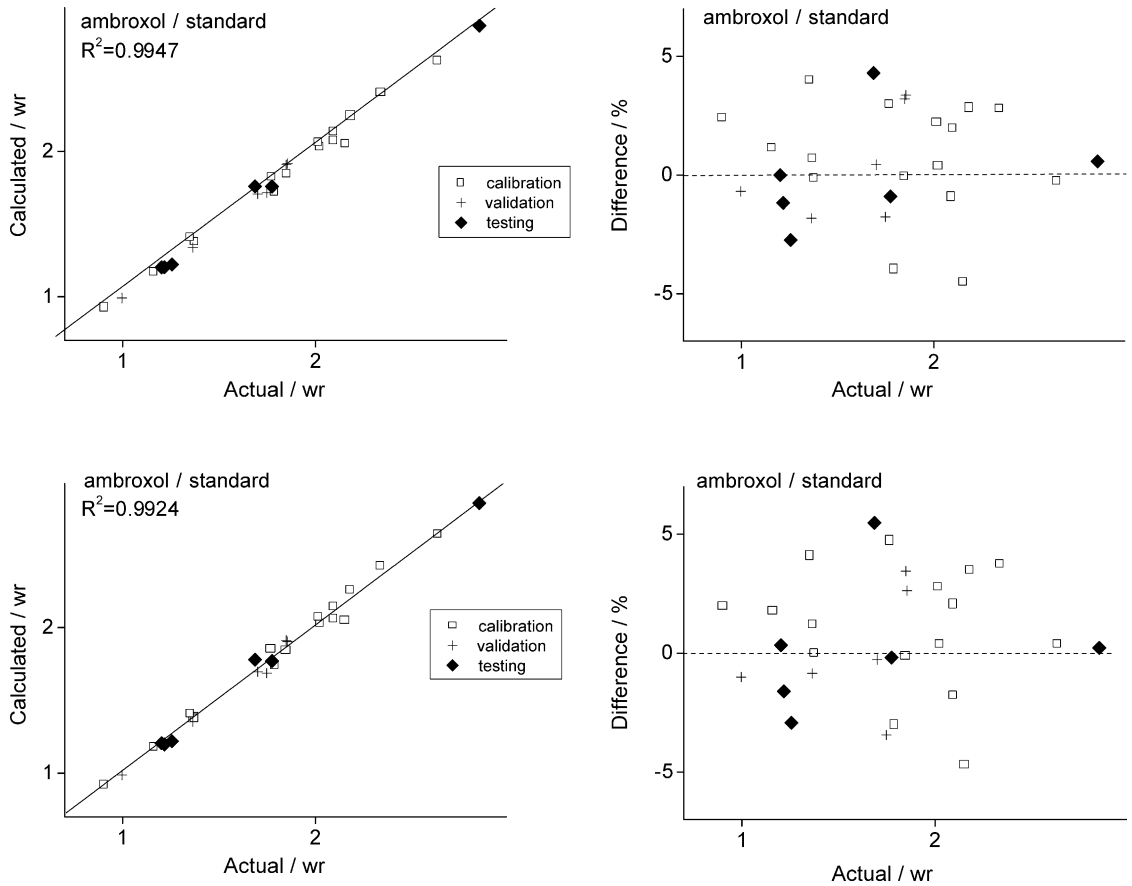

Fig. 4. Calibration curves and relative errors of ambroxol content with an internal standard added; PLS models constructed using spectra normalised by intensity at maximum (top) and integrated intensity (bottom) of the 2091 cm^{-1} potassium ferrocyanide band.

the Raman analysis equals 29.6 ± 0.4 mg per tablet, which correlates perfectly with the declared value of 30 mg of ambroxol for the studied medicine.

In the second step, potassium ferrocyanide was added as an internal standard to the mixtures and Raman spectra were recorded once more. New PLS models were constructed based on spectra normalised by the intensity at maximum and the integrated intensity of the 2091 cm^{-1} ferrocyanide line. The calibration curves and relative errors for ambroxol are shown in Fig. 4. The RSEP errors are a little higher for models constructed on the basis of normalised spectra. For 'unknown' samples, the RSEP error value for active ingredient quantification equals 3.0% in a case of normalisation by the intensity at maximum and 2.9% for normalisation by the integrated intensity of the internal standard line. Next, the commercial tablets with added potassium ferrocyanide were quantified. The mean amount of ambroxol found in the tablets equals 29.6 ± 0.6 and 30.8 ± 0.3 mg for the spectra normalised by the intensity at maximum and the integrated intensity, respectively.

It should be noticed that the Raman intensity scattered by the examined samples is rather low, so to obtain an acceptable signal to noise ratio, it was necessary to increase the number of interferogram accumulations. The poor signal-to-noise ratio could influence the quality of quantification, especially in the case of spectra normalisation. As mentioned before, all spectra were scanned 1024 times. For the new series of mixtures, the Raman spectra were recorded applying 128 scans only. It resulted in an increase of the RSEP values. The error calculated using the model constructed on the basis of unnormalised spectra equalled 2.7% for ambroxol quantification. The application of spectra normalised by the potassium ferrocyanide band resulted in errors approximately two times higher for the active ingredient determination. Quantification of the commercial tablets based on models constructed using spectra recorded eight times shorter gave only slightly worse results. The mean content of ambroxol in the studied preparation found from unnormalised spectra equals 30.2 ± 0.9, or 30.0 ± 0.6 and 29.4 ± 0.6 mg from the spectra normalised by the intensity at maximum and the integrated intensity of the internal standard line.

4. Conclusions

This study confirms the high potential of FT-Raman spectroscopy combined with PLS algorithm in the quantitative analysis of pharmaceuticals with low active ingredient content. The proposed procedure gives a level of

accuracy and precision comparable with that obtained for the tablet analysis with much greater active concentrations.

The proposed method is simple, as it does not require any special preparation of tablets for analysis. It could have potential applications in industry, as the official pharmacopoeial method of the ambroxol quantification in pharmaceuticals does not appear to be reported.

References

[1] S.G. Skoulika, C.A. Georgiou, Appl. Spectrosc. 57 (2003) 407.
[2] S.G. Skoulika, C.A. Georgiou, Appl. Spectrosc. 55 (2001) 1259.
[3] R. Szostak, S. Mazurek, Analyst 127 (2002) 144.
[4] T.H. King, C.K. Mann, T.J. Vickers, J. Pharm. Sci. 74 (1985) 443.
[5] J.M. Grange, N.J. Snell, J. Ethnopharmacol. 50 (1996) 49.