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Vibrational spectrometry for the assessment of active substance in metoprolol tablets: a comparison between transmission and diffuse reflectance near-infrared spectrometry

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Abstract

Near-infrared spectrometry (NIR) was used to quantify metroprolol succinate in controlled release tablets. Metoprolol tablets were made according to an experimental design using different strengths around a central strength of 47.5 mg per tablet. A comparison was made between NIR in the diffuse reflectance mode and the transmission mode. This showed that, although a narrower wavelength range was available in the transmission mode, predictions were much better for models based on transmission spectra than for models based on diffuse reflectance spectra. The main reason for this is that in the reflectance mode NIR spectromety is very sensitive to the inhomogeneity of the material, while in the transmission mode this problem is less severe. This is due to the larger volume of the material scanned in the transmission mode compared to that in diffuse reflectance. Spectra were taken before and after the tablets were stored under humid conditions. This allowed the final calibration models to be made more robust towards variations in the amount of water in the tablet. Different batches of metoprolol pellets and microcrystalline cellulose were used during the production of the tablets. This resulted in models that were more robust towards possible batch-to-batch differences in the main constituents.

Keywords: Diffuse reflectance; Multivariate calibration; NIR spectrometry; Tablets; Transmission

1. Introduction

Near-infrared spectrometry (NIR) is an analytical technique of growing pharmaceutical interest [1,2]. Although Herschel discovered the near-infrared region in 1800 [3], it was due to more recent developments in computer science that NIR became an analytical technique of interest. To be able to interpret the large amount of information present in NIR spectra, the combination of powerful computers and chemometric software is a prerequisite. To profit from NIR to its full extent, a chemometric approach based on multi-

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variate data analysis has to be used [4,5]. NIR offers a potentially fast, easy and, perhaps most importantly, non-invasive and non-destructive analytical technique. It is for these reasons that there is growing interest from the pharmaceutical industry in the analytical applications of NIR.

NIR analysis can be performed on raw materials (e.g. to establish purity), package materials, capsules [6], tablets [7] or in process streams [8]. Optical fibers [9] make it possible to collect spectra without exposing the instrumentation to the harsh conditions which can be present in, e.g., process streams. NIR can be used in both quantitative and qualitative analysis. One example of quantitative analysis is the determination of water in freeze-dried injection products [10,11]. The qualitative use of NIR has proven to be applicable for example to confirm the identity of blister-packed tablets for clinical trial supplies [12]. Clinical chemistry [13] is yet another field where NIR has proven its applicability. Quantification of serum glucose is only one of the applications studied within the field of clinical chemistry.

Presently, NIR equipment is available for both diffuse reflectance and transmission measurements. However, until recently the measurements on solid materials, e.g. tablets, were mainly performed in the diffuse reflectance mode [14,15]. As the reflectance mode only covers a small area of the material, this will cause problems if the homogeneity of the material cannot be assured. Therefore if NIR is to be used to quantify for example degradation products in tablets, the diffuse reflectance mode can give misleading results, as these products may not spread homogeneously. Also, for other quantification purposes, tablet inhomogeneity causes problems for NIR if applied in the diffuse reflectance mode. It is for these reasons that the ability to measure tranmission, even through solid materials such as tablets, would be of great benefit for the pharmaceutical industry. Transmission spectra represent a larger volume of the scanned sample, resulting in a better description of the material than diffuse reflectance spectra.

This paper describes the application of NIR in both the diffuse reflectance and transmission modes to measure metroprolol succinate in tablets. The content of metoprolol was quantified in bench-made tablets, which were similar to the controlled release Seloken ZOC® [16,17] tablets. Tablets were made with a strength in the range of ± 10% around 47.5 mg metoprolol succinate. To create a stable model, different batches of metoprolol and one of the other main constituents were included. Their amounts were varied according to a statistical design of the calibration set. Spectra were collected before and after storage of the tablets. Storage in humid conditions was done to make the model more robust towards variations in the amount of water in the tablet. Liquid chromatography (LC) was used as a reference method.

2. Materials and methods

2.1. Chemicals

Metroprolol (1-isopropylamino-3-[*p*-(2-methoxyethyl)-phenoxy]-2-propanol) succinate standards were produced by Astra Hässle AB. Acetonitrile, HPLC Grade S, was purchased from Rathburn (Walkerburn, UK). Sodium dihydrogen phosphate monohydrate and concentrated (85%) phosphoric acid were obtained from Merck (Darmstadt, Germany). Ethanol 95% (Spiritus Fortis) was procured from Kemetyl (Stockholm, Sweden). Concentrated (37%) hydrochloric acid was obtained from Kebo Lab AB (Spånga, Sweden).

2.2. Pellets

The tablets used for this study contained metoprolol succinate in the form of controlled release pellets. The diameter of the pellets was approximately 0.5 mm. Microcrystalline cellulose was used as a filler in the tablets and magnesium stearate as a lubricant.

2.3. Manufacturing of the tablets

A Korsch 106 rotating tabletting maching (Korsch, Berlin, Germany) equipped with six pairs of 9 mm punches with scores, was used for the

Table 1
Stated amounts of metoprolol succinate and microcrystalline cellulose in the different tablet batches

Batch	Metoprolol succinate (mg)	Pellet batch and content (%)	Metoprolol pellets (mg)	Microcrystalline cellulose (mg)
1	42.75	A (60.2)	71.01	137.89
2	45.12	A (60.2)	74.97	133.93
3	47.5	A (60.2)	78.9	130
4	49.88	A (60.2)	82.86	126.04
5	52.26	A (60.2)	86.79	122.11
6	42.75	B (59.9)	71.37	137.93
7	45.13	B (59.9)	75.34	133.96
8	47.5	B (59.9)	79.3	130
9	49.88	B (59.9)	83.27	126.03
10	52.25	B (59.9)	87.23	122.07

manufacture of the tablets. The microcrystalline cellulose and the metoprolol pellets were dry mixed for 4 min using a Kenwood mixer (Kenwood, UK). The amount of microcrystalline cellulose was varied between 122 and 138 mg per tablet to give a final weight of each tablet of approximately 209 mg as can be seen in Table 1. All tablets contained 0.209 mg magnesium stearate. The magnesium stearate was added to the mixture by sieving through a 0.7 mm sieve. Finally, mixing was performed for 2 min. Each batch comprised approximately 2000 tablets. By varying the compression force used during the manufacturing of the tablets, two different tablet heights (3.5 and 4 mm) were achieved. Close to 400 tablets of each height were collected.

2.4. Experimental design in the manufacturing of the tablets

During the production of the tablets, four parameters were varied. These parameters were:

(1) Five different contents of the drug, varying from 90–110%, in steps of 5%, of the stated amount of 47.5 mg per tablet (Table 1);

(2) Two different tablet heights, 3.5 or 4 mm, achieved through changes in the compression force;

(3) Two different lots of metoprolol pellets (A,B);

(4) Two different lots of microcrystalline cellulose.

This means that in total $5 \times 2 \times 2 \times 2 = 40$ different batches of tablets were produced. From every batch five tablets were individually stored in marked glass containers to allow tracing of the tablets during the whole study. During the study the containers were kept closed with a plastic stopper.

2.5. Tablet storage

The tablets initially contained approximately 3% of water. After collection of transmission and diffuse reflectance spectra of all tablets, they were stored, in their closed glass containers, in a climate room at 25°C and 80% relative humidity to force the tablets to take up water. The caps present on each vial allowed the uptake of small amounts of water. Each individual tablet was weighed before and after storage in the climate room. The average increase in tablet weight during storage was 5%. The calibration models are based on the spectra collected before and after storage and consequent water uptake. In this way changes in the water content were included in the calibration model as a separate design factor.

2.6. NIR apparatus

NIR spectra were collected on two different NIRSystems 6500 instruments (NIRSystems, Washington, DC). One instrument was equipped with a rotating drawer interface for diffuse reflec-

tance spectroscopy. The other instrument contained a special interface to enable transmission measurements on tablets. This device was developed by NIRSystems. A tablet holder was used in combination with this device. This holder was made in-house to fit the shape of the tablet. All transmission spectra were taken with the side without a groove closest to the incoming light. Turning the tablet upside down gave nearly identical transmission spectra. In total 40(batches) × 5(tablets per batch) × 2(before and after storage) = 400 transmission spectra were collected. Diffuse reflectance spectra were collected from both sides of each tablet. The side with the groove was measured first followed by the smooth side. Therefore, in the diffuse reflectance mode 400 × 2 = 800 spectra were collected. In both modes spectra were collected in the wavelength range 400–2500 nm with a resolution of 2 nm. This results in 1050 data points for every spectrum. However, the absorbance data obtained at wavelengths below 800 nm were not used, since the instrument performance is less accurate in this region.

2.7. Content of metoprolol succinate obtained by the reference method

After collection of all spectra from each individual tablet the reference LC analysis took place. First, each individual tablet in this study was weighed and transferred to a 50 ml volumetric flask. After adding 2.5 ml of water the flask was gently shaken in a shaking machine until the tablet had disintegrated. Then 25 ml of ethanol was added to the flask which was shaken again. After 30 min of gentle shaking, 5 ml of 0.1 M hydrochloric acid was added to the mixture and the flask was again shaken for 30 min. Finally 15 ml of 0.1 M hydrochloric acid was added and the flask was left to cool to room temperature. After dilution to volume with 0.1 M hydrochloric acid, the content of the flask was filtered through a glass microfibre filter. The first 10 ml of this filtrate was discarded. The resulting filtrate was diluted 1:20 with LC mobile phase. The samples were then analyzed by LC utilizing UV detection. The LC system consisted of a Varian 9012 pump (Varian Chromatography Systems, Walnut Creek, CA) in combination with a Waters 717 + autosampler from Millipore Corporation (Milford, MA). A Polygosil C8 5 μm 150 mm × 4.6 mm column from Hichrom Ltd. (Reading, UK) was used in combination with a Brownlee Newguard RP8 7 μm 15 mm × 3.2 mm precolumn from Applied Biosystems (Foster City, CA). Detection at 280 nm was carried out with a GBC LC 1210 UV/Vis Detector from GBC Scientific Equipment (Dandenong, Vic., Australia). As mobile phase a 27/100 (v/v) mixture of acetonitrile and pH 3.0 phosphate buffer (0.05 M) was used. Chromatograms were collected using a Perkin-Elmer Nelson 900 Series A/D interface (Cupertino, CA) and integration took place using the PE Nelson Access*Chrom 1.9 LC Data System (Cupertino, CA).

2.8. Statistical methods

Principal components analysis (PCA) and Partial least squares (PLS) calibrations were used according to Martens and Naes [18]. The developed models were evaluated by cross-validation using five cross-validation segments [19].

2.9. Software

The experimental design according to which the tablets were produced [20] was created with MODDE 2.1 software (Umetri AB, Sweden). All multivariate calibtrations were calculated using the Unscrambler 5.5 software (CAMO AS, Norway).

3. Results and discussion

3.1. Diffuse reflectance spectra of metoprolol succinate and metoprolol pellets

Fig. 1 shows diffuse reflectance spectra of metoprolol succinate (A), metoprolol pellets (B), and metoprolol tablets (C) respectively. The spectra shown are in the wavelength range 800–2500 nm. The effect on the spectra of making pellets of metoprolol succinate was clearly visible around

1400 nm, and at higher wavelengths, $\lambda > 2000$ nm. Moreover, a large increase in absorbance was observed when going from substance to pellets. From these spectra, and the spectra of intact tablets, the spectral information due to metoprolol pellets in the tablets could be extracted.

3.2. Calibration based on diffuse reflectance spectra

The diffuse reflectance spectra (Fig. 1) were digitized and collected to form an X matrix for PLS calibration with each spectrum as a row. The Y matrix contained the LC–UV detection reference values. The reflectance spectra of the tablets showed characteristics similar to the spectra of the metoprolol pellets around 1200 and 1400 nm. This is the second overtone region for the C–H stretch. From among all the spectra two obvious outlier spectra were removed.

For the modeling there was no significant difference in expressing the reference data as mg per tablet, mg per tablet weight or mg per table volume. The explained variance of the chosen response (mg active per tablet) was approximately 50% for two PLS components (Fig. 2), where the root mean square error of predictions (RMSEP), based on cross-validation, was 2.8 mg active per tablet, compared to the typical content of 47.5 mg

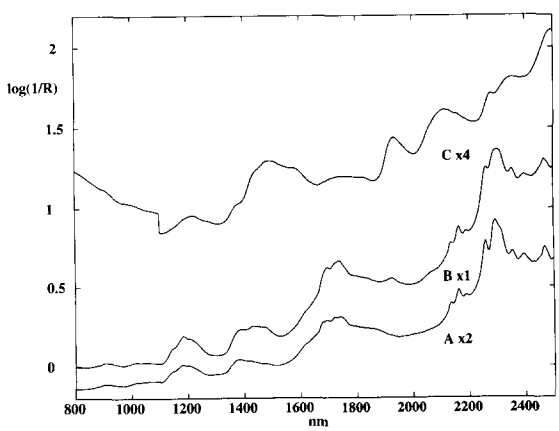

Fig. 1. Diffuse reflectance spectra of metorolol succinate powder (A), metoprolol pellets used to make the tablets (B), and one metoprolol tablet (C). Spectra A and B are obtained from a ≈ 1 cm layer of material. All spectra are obtained with a rotating cup.

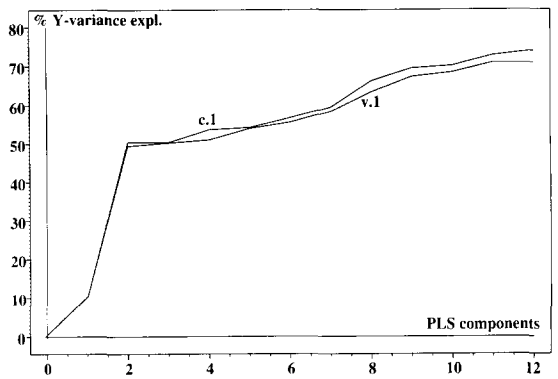

Fig. 2. The percentage of modelled Y variance for PLS components 1–12 when all spectra are included in the model (c.1), and calculated in PLS cross-validation by five successive groups of calibration spectra taken as test spectra (v.1) using diffuse reflectance spectra (800–2500 nm) as descriptors, and the amount of active substance in mg per tablet as the response.

per tablet. The explained variance by cross-validation slowly increased, without local minima, beyond 12 components and the RMSEP had a minimum after 11 compontents of 2.2 mg active per tablet. However, 11 components appear improbable for this calibration when the calibration is based on an experimental design with four factors. In this case the expected number of components would be approximately four, and possibly one or two additional components in order to compensate for nonlinearity. In the present case two of these four factors were clearly modeled. The minute increase in explained variance and prediction error beyond the second PLS component was interpreted as overfit. The calibration performance appeared independent of unit variance weighting X variables, and of spectral pretreatment with multiplicative scatter correction or second derivatives. The loading of the first component contained mainly offset (Fig. 3). According to the authors' experience this is often correlated with packing density, i.e. tablet hardness. This was confirmed by scores of PC 1 and 2 (Fig. 4), which show that the data are divided into two distinct groups according to the designed difference in tablet hardness. The loadings from PC 2 and 3 contain spectral information from metoprolol and water content respectively. Fig. 5

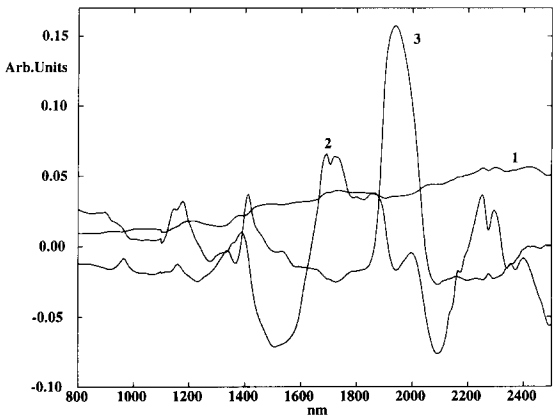

Fig. 3. Loadings for components 1–3 from PLS regression using NIR diffuse relflectance spectra.

shows the predictions, based on two principle components and cross-validation with five segments, versus the measured values of metoprolol content. The slope of this regression line is 0.5, which is far from the ideal of 1, with a standard error (SE) of 0.02. For the same regression line the intercept is 23, with a SE of 0.8.

3.3. Calibration based on transmission spectra

The digitized transmission spectra, as depicted in Fig. 6, were collected in the wavelength range

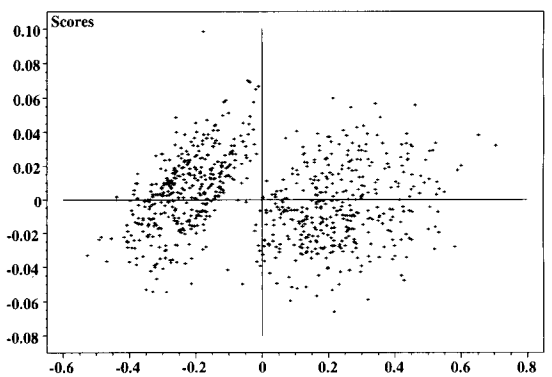

Fig. 4. Scores for PLS components 1 (abscissa) and 2 (ordinate) from PLS regression using NIR diffuse reflectance spectra (800–2500 nm) as descriptors and LC with UV detection data as the response. The cluster on the right-hand side represents tablets made at high compression force (tablet height: 3.5 mm), the cluster on the left-hand side contains the tablets made at lower compression force (tablet height: 4 mm).

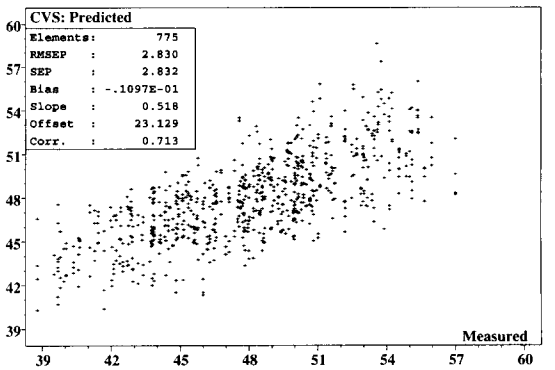

Fig. 5. The content of metoprolol succinate in tablets as calculated during the cross-validation (CVS) from the NIR diffuse reflectance spectra using four PLS components, versus the content determined by LC with UV detection at 260 nm. Abscissa and ordinate: mg metoprolol succinate per tablet.

400–2500 nm. However, as can clearly be seen in Fig. 6 only the region between 800 and 1350 nm can be used for calibration. In the region above 1350 nm too little light penetrates the tablet and therefore the detector signal becomes noisy. Below 800 nm, a high absorbance from the reference and other instrument properties make the spectra less dependable. The region where light penetrates the tablet includes 1200 nm where similarities with the spectra of the metoprolol pellets are visible. The useable part of the spectra (λ:800–1350 nm) was used to form an X matrix for multivariate calibrations.

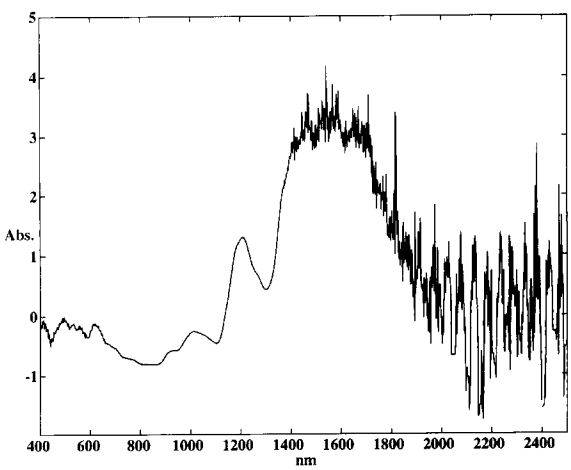

Fig. 6. An absorbance spectrum from the transmission through a 4 mm thick metoprolol tablet.

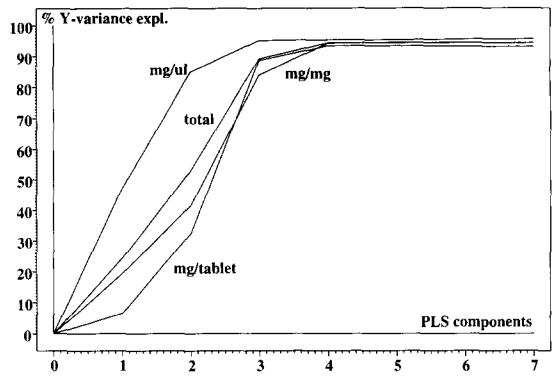

Fig. 7. The percentage of cross-validated Y variance for different measures of tablet content, related to tablet transmission spectra.

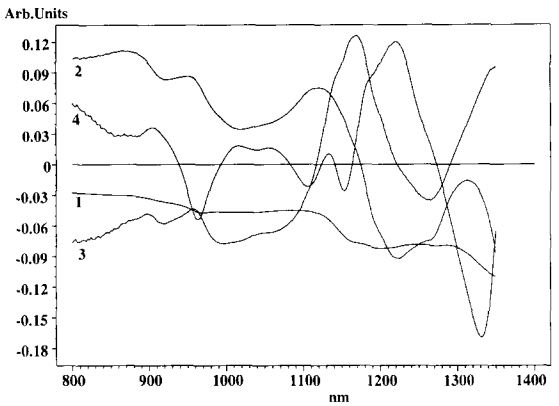

Fig. 8. The loadings for the PLS components 1–4 in the tablet transmission model. The wavelength range 800–1350 nm was used for the model.

PCA on all 400 spectra clearly detected 14 outliers. All outliers were collected on the same day and directly after each other. This is probably due to an operator mistake. These ouliers were excluded from the further calculations.

The results from the LC analysis were put as reference values in the $\boldsymbol{Y}$ matrix. The reference data were expressed as mg per tablet, mg per weight of the tablet and mg per volume of the tablet. During the calibration the $\boldsymbol{Y}$ matrix was weighted with 1/SDEV while the $\boldsymbol{X}$ matrix was presented to the model without weight correction. Fig. 7 shows that after four components, no matter how the Y values are expressed, up to 90% of the variance in the $\boldsymbol{Y}$ matrix is explained. However, if the $\boldsymbol{Y}$ matrix is expressed as mg per μl more than 90% is already explained after three components. If one looks at the RMSEP, which is based on cross-validation and gives a good indication of the prediction power [21], there is only a minor difference between calibration models based on mg per tablet and mg per μl, favouring the model based on mg per tablet. As mg per tablet is the least labor-intensive, and therefore probably the most accurate, way to express the $\boldsymbol{Y}$ matrix, this was chosen as being preferable. The use of multiplicative scatter correction did not significantly improve the models and was therefore not applied in the final model.

Fig. 8 shows the loadings for the first four components for this PLS model. The first component contained mainly offset, the second and third showed spectral features. The third component shows a characteristic peak in the loading plot around 1200 nm. This is the spectral area where information from the metoprolol pellets should be present.

Fig. 9 shows the scores for the first two components. In this Figure two clear groups (A and B) can be distinguished. These groups represent the spectra taken before (B) and after (A) storage of the tablets. So basically the first component takes care of the difference in water content in the tablets. However, a further subdivision into W, X,

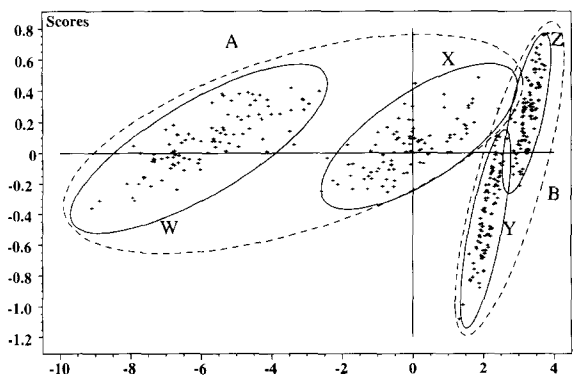

Fig. 9. The scores for components 1 (abscissa) and 2 (ordinate) in the tablet transmission PLS model. Tablets in group A (after storage) are thicker than those in group B (before storage) due to water uptake. The subdivisions W–Y (4 mm tablets) and X–Z (3.5 mm tablets) are caused by the different tablet compression forces which result in different tablet heights.

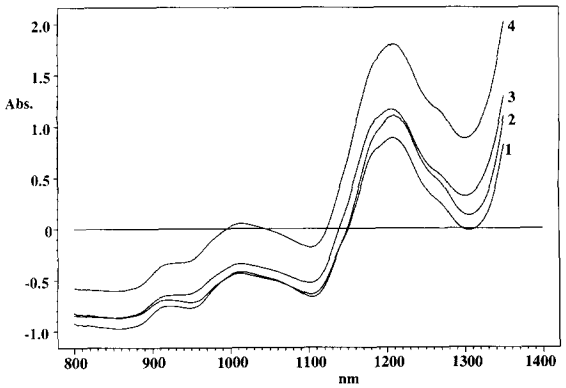

Fig. 10. Transmission spectra from tablets with different thickness before and after storage: (1) 3.5 mm, before storage; (2) 4 mm, before storage; (3) 3.5 mm after storage; (4) 4 mm, after storage.

Y, Z groups of this score plot is possible, (see Fig. 9). These groups represent the scores for the tablets made with different compression forces resulting in different thickness of the tablets. Groups W and Y contain the scores of the 4 mm tablets, while groups X and Z represent the scores of the 3.5 mm tablets. The first component seems to compensate for this effect. It is important to notice here that after uptake of water the tablets had become thicker. Spectra of tablets of 3.5 and 4 mm before and after storage are shown in Fig. 10.

Fig. 11 shows the predictions, which were based on cross-validation with five segments, versus the reference values of the metoprolol content per tablet. The PLS model was based on four components and explained 90% of the response. With this model the RMSEP equals 1.06 mg. The slope and offset for the line representing prediction versus LC measurement resulting from this model are respectively 0.93, SE 0.01, and 3.11 mg, SE 0.6.

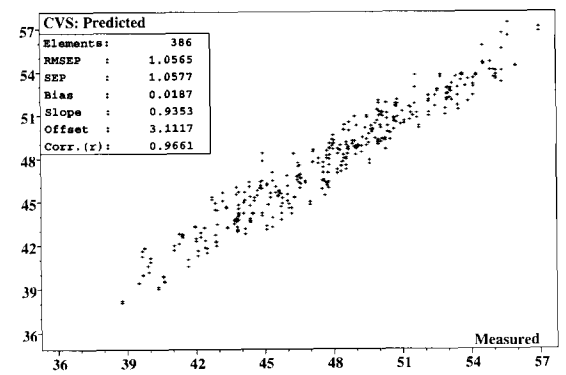

Fig. 11. The metoprolol content predicted with the tablet transmission PLS model versus the metoprolol content measure with the LC method based on cross-validation after four components. Absicissa and ordinate: mg metoprolol succinate per tablet.

4. Conclusions

The main conclusion from this study is that models based on transmission spectra give better prediction power, i.e. lower RMSEP, than models based on diffuse reflectance spectra. The major limitation of the transmission mode is that a large part of the wavelength range normally available (800–2500 nm) cannot be used for calibration purposes. However, the larger volume of the tablet scanned during transmission, compared to diffuse reflectance, fully compensates for this drawback. Therefore, the problem of inhomogeneity will remain related with reflectance spectroscopy. The wavelength window in which light penetrates tablets was dependent on the thickness of the tablet. However, the thickest metoprolol tablets studied, 4 mm, still gave enough spectral information to build reliable models. Complementary testing, the data of which are not included in this paper, showed that spectra from tablets up to 7 mm thick still possess a small wavelength window which lets through NIR radiation.

As the models were built on spectra before and after storage of the tablets in humid conditions, they should be robust against interference from water. Also, the use of different batches of metoprolol pellets and microcrystalline cellulose for the production of the tablets increased the stability of the final model.

During normal production of Seloken ZOC® it would have been impossible to make tablets in a wide range around the stated strength. Therefore, tailor-made calibration tablets were essential to build a calibration model. This means that the calibration model is based on bench-made tablets; therefore adaptation of the model for ZOC®

tablets obtained during production may be necessary. For content uniformity, where individual tablets are studied for their strength to control batch-to-batch production differences, transmission spectrometry should be the mode of choice. If transmission could be measured on-line for this purpose it would be very useful in pharmaceutical production.

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