ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 365–374

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Assay of effervescent tablets by near-infrared spectroscopy in transmittance and reflectance mode: acetylsalicylic acid in mono and combination formulations

P. Merckle, K.-A. Kovar *

Pharmazeutisches Institut der Universität Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

Received 2 April 1997; received in revised form 23 July 1997

Abstract

Near-infrared spectroscopy (NIRS) was used to determine acetylsalicylic acid (ASA) in three different effervescent tablet formulations. The nominal ASA concentrations were 14.9% in the single substance formulation (ASA Mono), 17.4% in the combination with ascorbic acid (ASA + C) and 8.7% in the combination with paracetamol and ascorbic acid (ASA Combi). In each case the tablet matrix was composed of seven excipients typical of effervescent tablets. All three formulations were measured as intact tablets in diffuse transmittance and reflectance and as powdered tablets in diffuse reflectance. Calibration was carried out by partial least square (PLS) regression of second derivative spectra. High-performance liquid chromatography (HPLC) was used as the reference method. The relative standard errors of calibration (RSEC) achieved for the three NIR methods were between 1.20 and 2.01% for ASA Mono, between 1.91 and 2.21% for ASA + C and between 2.41 and 4.50% for ASA Combi. The results obtained in transmittance mode were comparable with those obtained in reflectance mode, which is normally used in NIRS. In the test sets of ASA Mono and ASA + C relative root mean square (RRMS) values between 2.21 and 3.13% were obtained. The three NIR methods applied are thus suitable for the quantitative determination of ASA in effervescent tablets and have the advantage over HPLC of being rapid and simply carried out with little sample preparation; they are nondestructive and do not require any environmentally harmful reagents.

Keywords: Near-infrared spectroscopy; Diffuse reflectance; Diffuse transmittance; Intact tablets; Quantitative determination; ASA effervescent tablets; Partial least square

1. Introduction

Advances in software and improved NIR instruments have led to a wide application of NIRS in the pharmaceutical industry [1–4]. NIRS is a rapid, nondestructive method suitable for the analysis of raw materials and finished products. The weak molecular absorbance of the molecules in the NIR range makes it possible to carry out determinations without sample preparation directly on the end-product. Hence, NIRS is ideal for the analysis of tablets and solid pharmaceutical formulations [5]. Until now the quantitative

* Corresponding author. Tel.: + 49 7071 2972470; fax: + 49 7071 292470; e-mail: karl-arthur,kovar@uni tuebingen,de

0731-7085/98/$19.00
PII S0731-7085(97)00194-5

determination of active substances in solid dosage forms has primarily been performed in diffuse reflectance. Powdered samples yielded results comparable in accuracy with those of the reference method employed, but usually on formulations with a high content of active substance or a simple excipient matrix [6–10]. The nondestructive nature of NIRS is only exploited to the full when determinations are carried out on intact tablets. This opens up new fields of applications, such as determination of degradation products [11], qualification of clinical batches [12], determination of film-coated tablet parameters [13] and quantitative in-process determinations, which make it possible to intervene in the running production process. Quantitative determinations on intact tablets have not been described very often until now [14,15]. The simultaneous determination of several active substances with only one measurement is a further advantage of NIRS. This has been demonstrated on pure active substance mixtures [16], in liquid preparations [17] and in laboratory mixtures [18], but not on intact tablets with a complex excipient matrix. Quantitative determinations on solid preparations have recently become possible in the transmittance mode. Using a special accessory it was possible to determine the active substance in a powdered mixture with similar results to those obtained by diffuse reflectance [19]. Recently commercial NIR transmittance spectrometers for determinations on whole tablets have become available. Hence, it is to be expected that simple and rapid quantitative determinations in transmittance can now also be carried out directly on the endproduct. This has not yet been described in the literature.

This paper describes the quantitative determination of acetylsalicylic acid (ASA) in three different effervescent tablet formulations using NIRS in reflectance and transmittance modes. The formulations contained ASA alone, ASA in combination with ascorbic acid, and ASA in combination with ascorbic acid and paracetamol, and their compositions corresponded to those of commercial preparations. HPLC was used as the reference method. The determinations were carried out on intact tablets in transmittance and reflectance mode and on powdered tablets in reflectance mode. The evaluation of the results of the individual formulations was used to demonstrate the strengths and weaknesses of the three different NIR methods.

2. Materials and methods

2.1. Instruments

NIR reflectance data were collected using a NIRSystems, Inc. Model 5000 NIR spectrophotometer (Silver Spring, MD, USA) for intact tablets and NIRSystems, Inc. Model 6500 for powdered tablets. In both cases data were collected over the 1100–2500 nm wavelength range. NIR transmittance data were collected using a NIRSystems, Inc. Model 6500 spectrophotometer equipped with an experimental tablet transmittance module; data were collected over the 700–1300 nm wavelength range. Near-infrared spectral analysis software (NSAS, NIRSystems Inc.) was used for preprocessing and analysis of spectra.

2.2. Samples

The study was carried out using ASA effervescent tablets having a composition identical to that of three preparations (ASS-ratiopharm®, ASS + C-ratiopharm® and ASS-Kombi gegen Fieber und Schmerzen ratiopharm®) marketed by ratiopharm, Germany. The first formulation (ASA Mono) contained ASA as the only active substance, the second (ASA + C) contained ASA in combination with ascorbic acid and the third (ASA Combi) contained ASA in combination with paracetamol and ascorbic acid. The excipients used were sodium hydrogen carbonate, mannitol, sodium dihydrogen citrate, citric acid, sorbitol, polyvinylpyrrolidone, lactose, lemon aroma, adipic acid and saccharin sodium. For the purpose of calibration, effervescent tablets containing doses of 80, 90, 100, 110 and 120% related to the nominal ASA concentration of each formulation were prepared. This covers the range usually required by the approval authorities. The nominal concentrations of ASA were 14.93% in ASA Mono, 17.39% in ASA + C and 8.7% in

ASA Combi. The average ascorbic acid content was 5.8% for ASA + C and 8.7% for ASA Combi, the paracetamol content of ASA Combi was 5.8%. In an additional batch the content of ascorbic acid and paracetamol respectively was varied. The excipients remained the same. The tablets were 2 cm in diameter and 2.9 mm thick, they weighed about 2200 mg. A calibration set of 30 tablets for each formulation was used for calibration. The calibration was checked using for each formulation an independent test set of ten tablets likewise covering the whole calibration range. The intact and powdered tablets were measured with the respective NIR instruments and then analysed by HPLC as the reference method.

2.3. Transmittance measurements

The NIR radiation was directed onto the tablet from above. The detector was positioned directly below the tablet. A Teflon disc 1.59 mm thick was used and the sensitivity of the detector optimised on it. For each tablet spectra were recorded at both sides. Each spectrum was the average spectrum from 32 scans.

2.4. Reflectance measurements

When measurements were carried out on intact tablets the NIR radiation impinged on a centred tablet. Spectra were recorded from the front and back surface of each tablet. The measurements of the powdered tablets were made in a sample cup. The samples were measured twice with a horizontal rotation of 180°. The spectrum of a diffusely reflecting ceramic disc served as the reference spectrum in both cases. Both methods yielded a spectrum representing an average spectrum from 16 scans.

2.5. Calculations

A partial least square (PLS) regression was used to relate the spectroscopic data to the ASA concentration [20]. PLS is a factor-based procedure which makes it possible to process all the data from a full spectrum. PLS attempts to explain as much of the variation in the dependent variable as possible, using only the relevant factors contained in the spectral data. The data of the calibration set are used to develop the PLS model. Here the model was constructed by cross-validation of the second-derivative spectra. This model can then be used to predict the concentrations corresponding to the spectra obtained from the test set. The standard error of calibration (SEC) was used for the evaluation of the calibration and it was referred to the average ASA concentration of the calibration set in the form of the relative standard error of calibration (RSEC).

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{n} (C_{\mathrm{REF}} - C_{\mathrm{NIR}})^2}{n - m - 1}} \ [\%\ \mathrm{w/w}]$$

$$\mathrm{RSEC} = \frac{100 * \mathrm{SEC}}{C_{\mathrm{REF}}} \ [\%]$$

The SEC is the standard deviation of the differences between the concentration values of the reference method (C_{REF}) and the calculated concentrations obtained by NIRS (C_{NIR}), whereby n represents the number of samples in the calibration set and m the number of factors used. The correlation coefficient (CC) is another criterion employed. A value close to 1 reveals a high correlation between the values obtained by the reference method and the values calculated by the NIR model. The calibration is then checked using the samples of the test set on the basis of the root mean square error (RMS) which is again referred to the average concentration of the reference values of the test set in the form of the relative RMS (RRMS). The calculation of the RMS is carried out like that of the SEC, apart from the inclusion of the factors.

$$\mathrm{RMS} = \sqrt{\frac{\sum_{i=1}^{n} (C_{\mathrm{REF}} - C_{\mathrm{NIR}})^2}{n - 1}} \ [\%\ \mathrm{w/w}]$$

$$\mathrm{RRMS} = \frac{100 * \mathrm{RMS}}{C_{\mathrm{REF}}} \ [\%]$$

2.6. Reference method

The HPLC analysis of ASA in each tablet was performed using a validated USP XXIII method

(Aspirin Effervescent Tablets for Oral Solution). The results were reported as a percentage of the concentration of the active substance. The relative standard deviation of the method was 2%.

3. Results and discussion

3.1. Spectra recorded in diffuse reflectance and diffuse transmittance

Diffuse reflectance spectra are generally recorded over the wavelength range 1100–2500 nm. When tablets were measured by diffuse transmittance a limited wavelength range of 700–1300 nm was employed since the absorbance of the tablets became too high at wavelengths over 1300 nm. This wavelength range primarily includes the third overtone region where the absorptions are considerably weaker than at longer wavelengths. However, this disadvantage is compensated-for by the fact that in transmittance measurements the effective path length is longer than it is in diffuse reflectance measurements where measurements are carried out at the surface only. In the transmittance spectra (Fig. 1a) the bands are stronger than in the reflectance spectra (Fig. 1b), which exhibit bands with lower resolution and a steeply ascending baseline. In the overlapping range from 1100 to 1300 nm (Fig. 1c), it can be demonstrated that the positions of the band maxima in transmittance are in agreement with those in reflectance. By forming the second derivative, baseline shifts are equalised and the resolution of the overlapping active substance bands is improved. The minima now appear at the same positions as the maxima in the untransformed spectra (Fig. 2a, b).

3.2. Assay of ASA in the three formulations

In order to construct a robust calibration the tablets of the individual formulations were recorded by NIRS and HPLC over several weeks. The variations in the NIR and reference measurements were thus included in the calibration. In addition, the concentrations of ascorbic acid and paracetamol respectively,were varied in one batch each of ASA + C and ASA Combi in order to investigate the effects on the determination of ASA. When constructing the calibration curve (using PLS it is a straight line) it turned out that for the complex effervescent tablet mixtures PLS

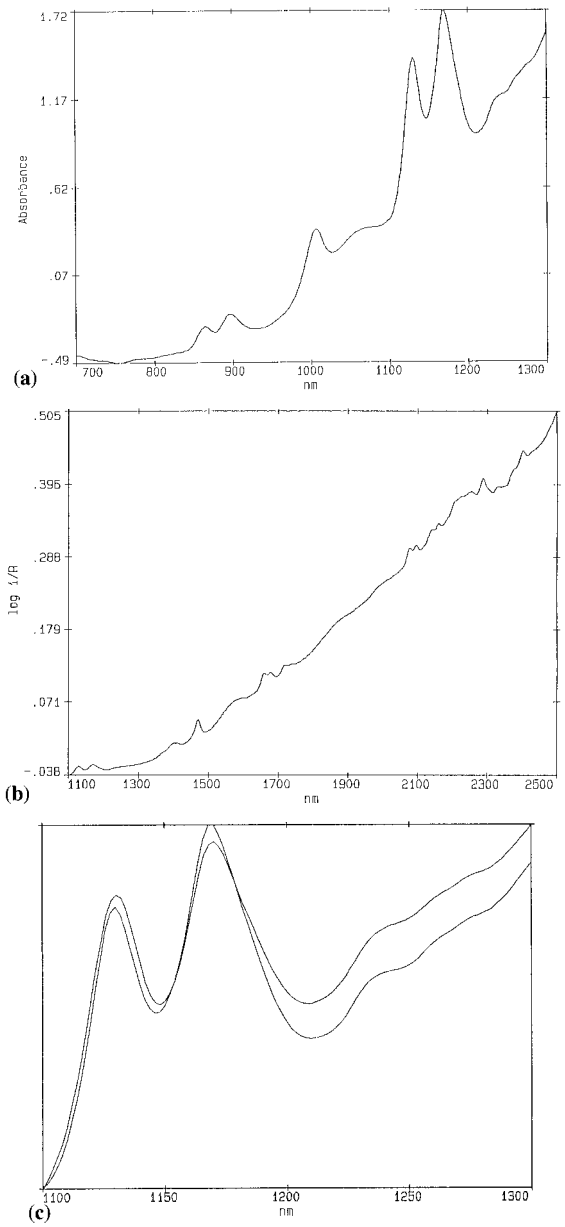


Fig. 1. Near-infrared spectra of intact ASA Mono effervescent tablets in diffuse transmittance (a) and diffuse reflectance (b). Comparison of spectra in the region of 1100–1300 nm shows the identical position of the band maxima.

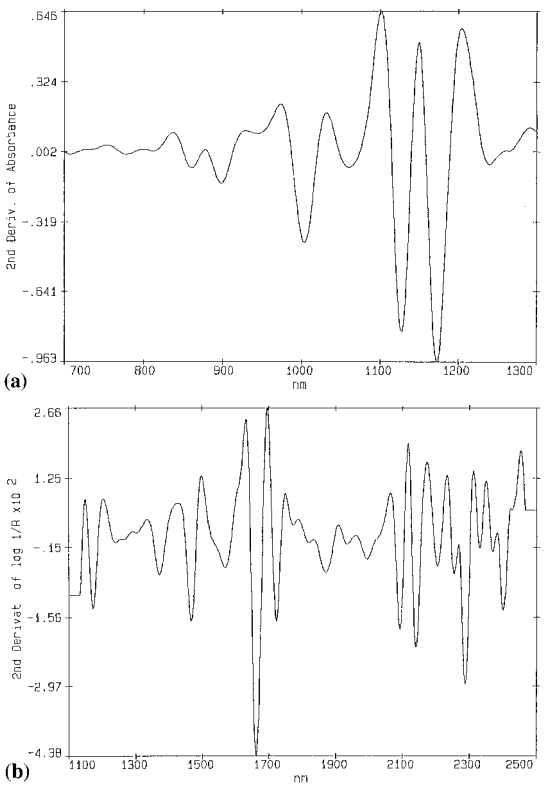


Fig. 2. Second derivative of near-infrared spectra of ASA Mono effervescent tablets in diffuse transmittance (a) and diffuse reflectance (b).

regression was superior to multiple linear regression. Hence, only the results of PLS calibrations are presented here. The choice of the number of factors for description of the PLS model was carried out taking the SEC and the spectral variation into account. If too many factors are included the calibration only apparently improves, because simultaneously it becomes less robust. The three methods yielded RSEC values between 1.20 and 2.01% for ASA Mono, between 1.91 and 2.21% for ASA + C and between 2.41 and 4.50% for ASA Combi (Table 1). Since the calculation of the deviations depends on the number of factors chosen, the RSEC were calculated for the first ten factors as the mean value for the three methods, in order to be able to compare the formulations better (Fig. 3). Hence it follows that ASA could be quantified better in ASA Mono and ASA + C than it could in ASA Combi. The differences between ASA Mono and ASA + C were small. Already with factor 2 the differences in RSEC values were less than 1% with ASA + C yielding the better results. From this it can be concluded that ascorbic acid, at the concentration present here, does not have any great influence on the quantification of ASA. This was also confirmed on comparing calibrations with and without variations in the ascorbic acid content. In contrast to this the combination of ASA with ascorbic acid and paracetamol in ASA Combi had an adverse effect on the calibration of ASA. Part of this is attributable to the lower concentration of ASA, in addition paracetamol and ASA have aromatic basic structures so that there is superimposition of the aromatic bands. However, an RSEC of 2.4% and a correlation coefficient of >0.99 (Table 1) prove that it is still possible to calibrate ASA in combination formulations such as ASA Combi in a manner which is comparable with the reference method.

In the next step the constructed calibrations were checked using the tablets of the test sets in order to exclude the possibility of overfitting. These tablets were measured at a later point in time than those of the calibration sets. The spectral information of the test set was now used to predict the ASA concentrations of the formulations on the basis of the constructed calibration and was compared with the reference data subsequently determined by HPLC. The RRMS was between 2.21 and 3.13% for ASA Mono, between 2.62 to 3.13% for ASA + C and 3.73 to 7.83% for ASA Combi (Table 1). The correlation coefficients for all three formulations, with one exception for ASA Combi, lay between 0.981 and 0.997 and revealed a good agreement between the values calculated by NIRS and those determined by the reference method over the whole calibration range. The comparatively poorer result obtained for ASA Combi can primarily be attributed to the extreme variation in the paracetamol content in the composition of the additional test batches in which paracetamol was included at 120% and ASA at 80% of the expected levels. This variation, which would never occur in practice,

Table 1
Statistical summary

	Transmittance	Reflectance	
	Intact tablet	Intact tablet	Powdered tablet
ASA Mono			
Calibration set			
Number of used factors	6	6	6
SEC (% w/w)	0.29	0.26	0.17
RSEC (%)	2.01	1.79	1.20
Coefficient of correlation	0.991	0.993	0.997
Test set			
RMS (% w/w)	0.45	0.32	0.37
RRMS (%)	3.13	2.21	2.56
Coefficient of correlation	0.987	0.99	0.991
ASA+C			
Calibration set			
Number of used factors	3	5	3
SEC (% w/w)	0.36	0.36	0.31
RSEC (%)	2.21	2.20	1.91
Coefficient of correlation	0.989	0.989	0.99
Test set			
RMS (% w/w)	0.52	0.43	0.47
RRMS (%)	3.13	2.62	2.87
Coefficient of correlation	0.981	0.988	0.997
ASA Combi			
Calibration set			
Number of used factors	3	6	4
SEC (% w/w)	0.26	0.37	0.20
RSEC (%)	3.11	4.50	2.41
Coefficient of correlation	0.988	0.976	0.993
Test set			
RMS (% w/w)	0.32	0.63	0.30
RRMS (%)	4.02	7.83	3.73
Coefficient of correlation	0.991	0.965	0.996

led to the greatest deviations in the analysis of the test sets.

If the RRMS of 2.56% obtained for diffuse reflectance of powdered ASA Mono tablets was compared with the determination of ascorbic acid in effervescent tablets (nominal content 22.88%) carried out by Blanco et al. [7], the (relative) validation error of 2.85% found there is of a similar order of magnitude. The determination of two active substances (nominal contents 14.8 and 1.48%) in an excipient mixture carried out on laboratory powder samples by Corti et al. [18] also provided comparable results. A relative standard error of 2.5–6.7% was achieved in the test sets.

3.3. Comparison of the various NIR methods

Carrying out measurements on intact and powdered tablets of different compositions makes it possible to recognise the strengths and weaknesses of the three NIR methods used. The NIR spectrum is influenced by physical properties, such as particle size, particle size distribution, crystal structure, homogeneity and packing density. Hence, an NIRS calibration should be carried out with samples whose physical properties correspond to those of the samples to be determined. This is only the case for tablets from the production process; but these do not possess the calibra-

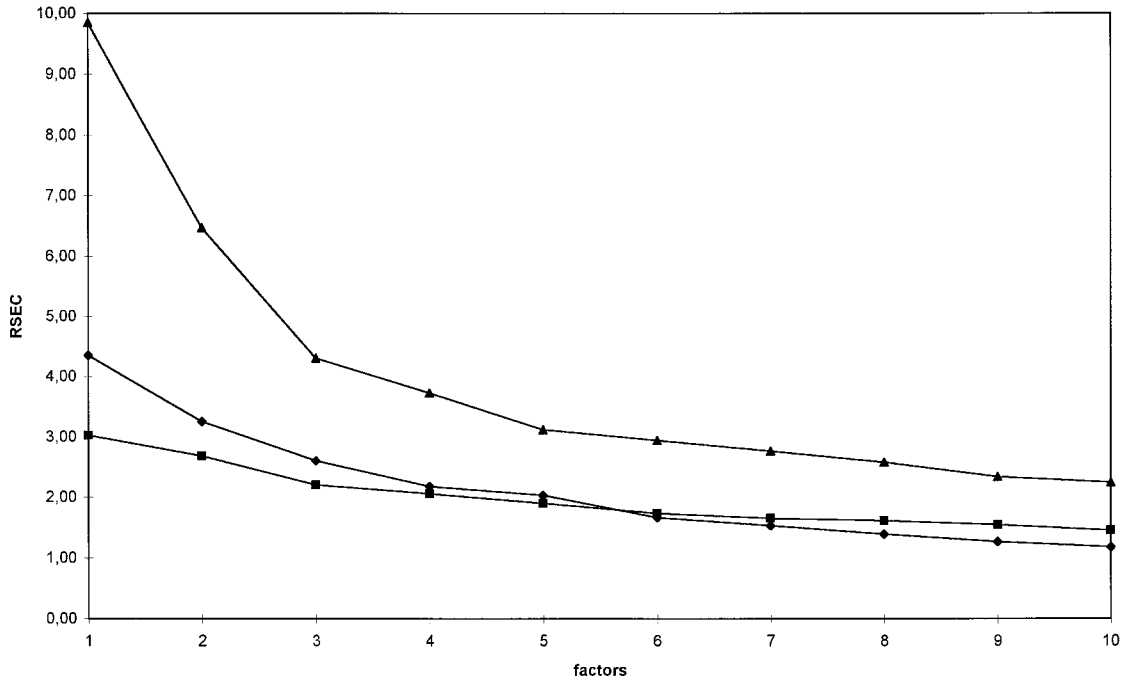


Fig. 3. Comparison of the average relative standard error of calibration by three methods at varying factors for all three formulations: ASA Mono (◆), ASA + C (■), and ASA Combi (▲).

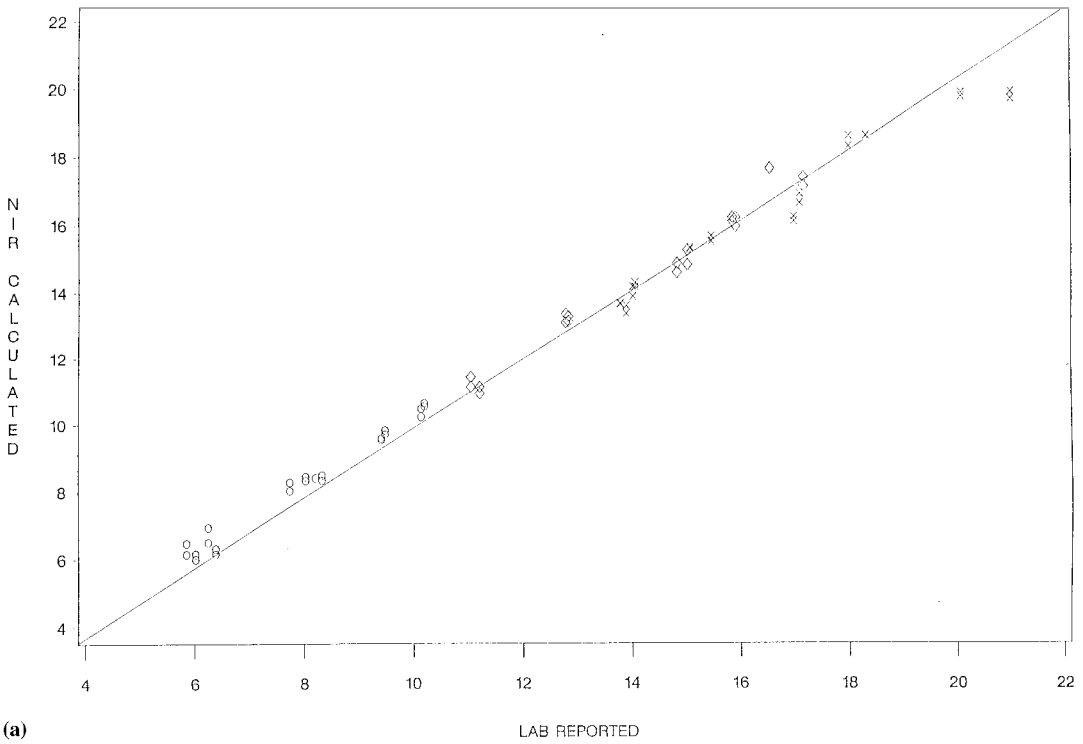
tion range necessary for construction of a calibration curve.

The construction of a calibration using laboratory powder mixtures represents a simple possibility of obtaining a sufficient calibration range. However, this calibration can be used for the assay of the production tablets to a limited extent only, because when using powdered mixtures typical properties from the production process are missing. For this reason extrapolations to powdered tablets are only of limited utility. Molt [15] found that this led to a systematic error of 2% w/w (nominal content 83%).

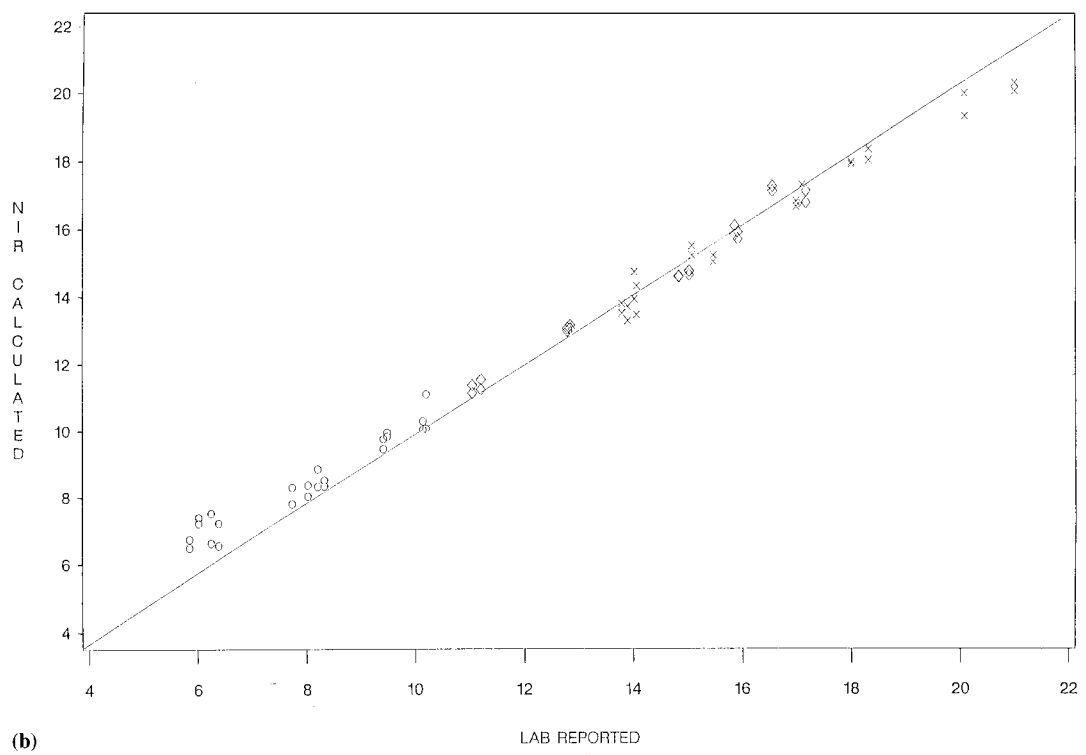
The spiking of powdered production tablets with excipients or active substances represents a further common possibility of constructing a sufficient calibration range. Using this process the physical properties of the production tablets are largely included [10].

By constructing a calibration of intact tablets over the desired calibration range, the best possible agreement of physical properties with the tablets from the production process is achieved. This requires a greater effort than a calibration of powdered tablets. However, once calibration is completed the NIR measurement of intact tablets offers the advantage that sample preparation is completely unnecessary. The quantitative determination can be carried out in less than one minute and is not dependent on the skill of the person carrying out the determination. The tablets remain undamaged and are available for further use. Pulverisation of the tablets is the only preparation step necessary for the measurement in diffuse reflectance of powders. Here, it is necessary to ensure that uniform particle size distribution of the samples is maintained if reproducible results are to be obtained.

All three NIR methods revealed good agreement between the data calculated by NIRS and those reported by HPLC (Fig. 4). To compare the three NIR methods, an analysis of variance test (ANOVA) was carried out. For this the differences of the ASA values calculated by NIR and


NIR CALCULATED
LAB REPORTED
4
6
8
10
12
14
16
18
20
22

(a)


NIR CALCULATED
LAB REPORTED
4
6
8
10
12
14
16
18
20
22

(b)

Fig. 4.

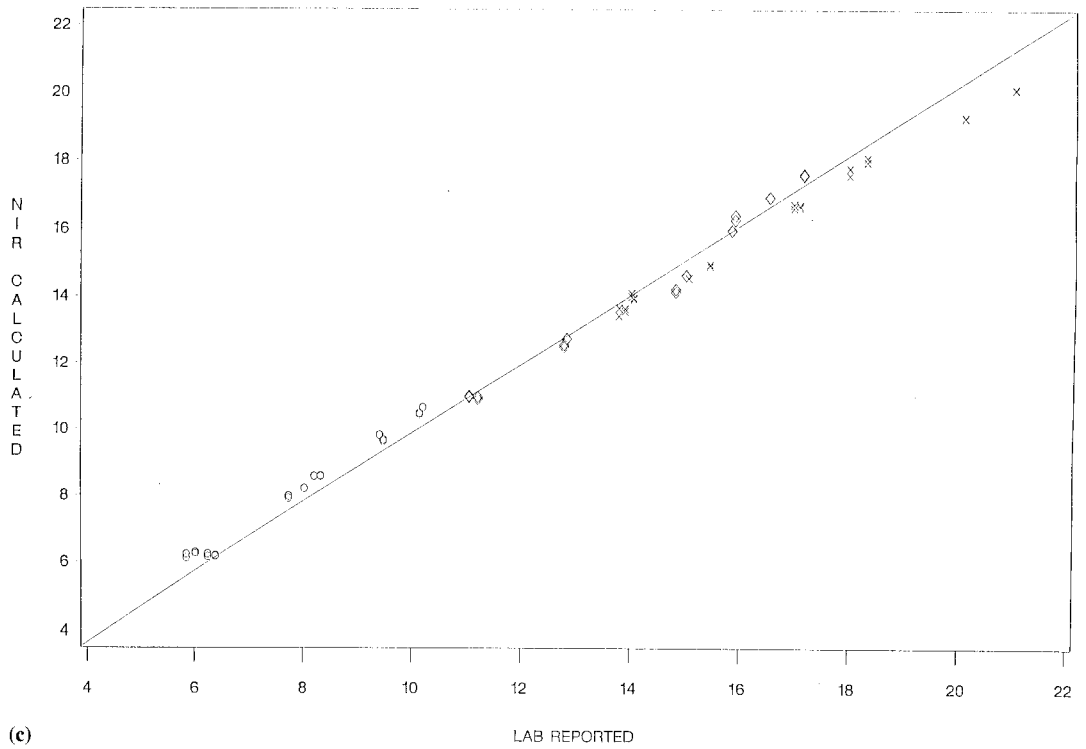


Fig. 4. Correlation of the NIR methods for the three preparations ASA Mono (open stars), ASA + C (Xs), and ASA Combi (open circles) by NIR in % w/w: transmittance of intact tablets (a), reflectance of intact tablets (b), and reflectance of powdered tablets (c). The line represents a theoretical perfect correlation between the reported HPLC and calculated NIR values.

HPLC were analysed for each tablet of the test sets. For ASA Mono and ASA + C there was no significant difference between the three methods at the $p = 0.05$ level. The results of the transmittance measurement were hence comparable with those for the two reflectance methods. For ASA Combi transmittance measurements of intact tablets and reflectance measurements of powdered tablets yielded better results than measurements in diffuse reflectance of intact tablets.

The ASA Combi example showed that the measurement in diffuse reflectance at the surface of tablets can be subject to interference. Tablet parameters, such as hardness or inhomogeneities can lead to differing scattering properties of the tablet surface. So it was possible to detect differences between the spectra of the upper and lower surfaces of the tablets; these differences indicate that the upper and lower punches of an eccentric tabletting press exert different pressing forces or that there is stratification of the particle size of tabletting mixtures or substances of the internal and external phase. Lodder and Hieftje [14] minimised this effect by recording the whole surface of the intact tablets in diffuse reflectance. However, a higher quantity of light interacting with the sample can be obtained using transmittance measurement. Fong and Hieftje [19] were able to obtain results with the diffuse transmittance of a powder mixture of several similar active substances that were comparable to those obtained by diffuse reflectance. This result could also be confirmed in the case of ASA Combi for the recording of the spectra of intact tablets by diffuse transmittance as compared to diffuse reflectance with powders. This shows that surface effects of intact tablets are minimised by measurement in diffuse transmittance. The longer effective

path length leads to a large number of scattering and diffuse reflectance processes within the intact tablet and thus improves the quantitative determination.

A constant layer thickness is a necessary requirement for quantitative analysis using transmittance measurements. The influence of the tablet height on the transmittance spectrum was therefore tested. A reduction in the tablet height by rubbing tablets with a sandpaper led to a shift of the baseline of the spectrum. The absorbance was decreased over the whole range. When the spectra of the tablets used for calibration were evaluated, it was not possible to detect any linear dependence between the tablet height and the content measured within the differences in height of up to 1% that occurred. This can be attributed to the fact that the packing density is increased when the height of the tablet is lower.

In measurements of powdered tablets in diffuse reflectance a reproducible particle size distribution that is of paramount importance for the quality of the result. If the calibration is carried out without spiking, so that the calibration samples correspond to the tablets to be determined in all physical properties, this method leads to the most reproducible results.

Accordingly, diffuse reflectance can also be used for granules whose properties correspond to those of production samples. This is shown by the results of Dreassi et al. [10], where the two active substances contained in a granule mixture (nominal concentrations 6.13 and 1.06%) could be determined with relative standard errors of 1.23 and 6.13%.

4. Conclusions

The three NIRS methods applied are suitable for the quantitative determination of active substances in complex effervescent tablet mixtures. When measurements are carried out on intact tablets, particularly by diffuse transmittance, a large number of physical and chemical properties are recorded spectrally, thus producing a characteristic overall picture of the tablet. This means that the full potential of NIRS can be exploited: rapid and simple measurement, noninvasive and nondestructive, no sample preparation and no consumption of environmentally harmful reagents. In addition to quantitative determination new aspects are opened for quality control, e.g. in-process control and process validation. However, the good results obtained in the assay of intact tablets require elaborate calibration. It is necessary to prepare tablets equivalent in properties to production tablets, but with differing active substance concentrations and to determine these using a reference method.

References

[1] E.W. Ciurczak, Appl. Spectrosc. Rev. 23 (1987) 147–163.
[2] J.K. Drennen, R.A. Lodder, in: G. Patonay (Ed.), Advances in Near-Infrared Measurements, JAI Press, Greenwich, CT, 1993, pp. 93–112.
[3] P. Corti, E. Dreassi, S. Lonardi, Il Farmaco 48 (1993) 3–20.
[4] K.M. Morisseau, C.T. Rhodes, Dev. Ind. Pharm. 21 (1995) 1071–1090.
[5] J.D. Kirsch, J.K. Drennen, Appl. Spectrosc. 30 (1995) 139–174.
[6] S.P. Jacobsson, M. Carlson, U. Jönsson, G. Nilsson, J. Pharm. Biomed. Anal. 13 (1995) 415–417.
[7] W. Plugge, C. van der Vlies, J. Pharm. Biomed. Anal. 11 (1993) 435–442.
[8] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. de la Pezuela, Talanta 40 (1993) 1671–1676.
[9] E. Dreassi, G. Ceramelli, P. Corti, P.L. Perruccio, S. Lonardi, Analyst 121 (1996) 219–222.
[10] E. Dreassi, G. Ceramelli, P. Corti, M. Massacessi, P.L. Perruccio, Analyst 120 (1995) 2361–2365.
[11] J.K. Drennen, R.A. Lodder, J. Pharm. Sci. 79 (1990) 622–627.
[12] M.A. Dempster, B.F. MacDonald, P.J. Gemperline, N.R. Boyer, Anal. Chim. Acta 310 (1995) 43–51.
[13] J.D. Kirsch, J.K. Drennen, J. Pharm. Biomed. Anal. 13 (1995) 1276–1281.
[14] R.A. Lodder, G.M. Hieftje, Appl. Spectrosc. 42 (1988) 556–558.
[15] K. Molt, F. Zeyen, E. Podpetschnig-Fopp, Pharm. Ind. 58 (1996) 847–852.
[16] K. Molt, M. Engelkraut, GIT Fachz. Lab. 33 (1989) 1311–1313.
[17] P. Dubois, J.-R. Martinez, P. Levillain, Analyst 112 (1987) 1678–1679.
[18] P. Corti, E. Dreassi, R. Ballerini, S. Gravina, Pharm. Acta Helv. 65 (1990) 189–193.
[19] A. Fong, G.M. Hieftje, Appl. Spectrosc. 49 (1995) 486–492.
[20] H. Martens, T. Naes, Multivariate Calibration. Wiley, New York, 1991.