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Comparison of FT-NIR transmission and UV–vis spectrophotometry to follow the mixing kinetics and to assay low-dose tablets containing riboflavin

C. Bodson [a,*], W. Dewé [b], Ph. Hubert [c], L. Delattre [a]

[a] *Laboratory of Pharmaceutical Technology, University of Liège, 1 Avenue de l'hôpital, 4000 Liège, Belgium*
[b] *Lilly Services S.A., 11 Rue Granbonpré, 1348 Mont-Saint-Guibert, Belgium*
[c] *Laboratory of Analytical Chemistry, University of Liège, 1 Avenue de l'hôpital, 4000 Liège, Belgium*

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Abstract

For several years, near-infrared spectroscopy (NIRS) has become an analytical technique of great interest for the pharmaceutical industry, particularly for the non-destructive analysis of dosage forms. The goal of this study is to show the capacity of this new technique to assay the active ingredient in low-dosage tablets. NIR spectroscopy is a rapid, non-destructive technique and does not need any sample preparation.

As an example, a binary mixture of microcrystalline cellulose and riboflavin was used to prepare tablets of different weights by direct compression. A prediction model was built by using a partial least square regression fit method. The NIR assay was performed by transmission. The results obtained by NIR spectroscopy were compared with a conventional UV–vis spectrophotometry method.

The study showed that tablets can be individually analysed by NIR with high accuracy. It was shown that the variability of this new technique is less important than that of the conventional method which is the UV–vis spectrophotometry.

Keywords: FT-NIR transmission; PAT; Tablet assay; Validation; UV–vis assay; Solid dosage form; PLS model

1. Introduction

Currently, we hear a lot about the Food and Drug Administration's process analytical technology (PAT) initiative which is a collaboration effort with industry to facilitate the introduction of new and efficient manufacturing technologies.

PAT are systems for design, analysis, and control of manufacturing processes, based on timely measurements of critical quality and performance attributes of raw and in-process materials and products, to assure high quality of products at the completion of manufacturing (http://www.fda.gov/cder) [1]. PAT includes scientifically based process design that identifies key measurements of product quality and the critical process variables that affect them, appropriate measurement devices, statistical information technology tools, and feedback process control strategies that work together to ensure production of final products with the desired quality.

Several vibrational spectroscopy techniques are used for the application of PAT in the on-line monitoring of the pharmaceutical process.

For several years, near-infrared spectroscopy (NIRS) has become an analytical technique of great interest for the pharmaceutical industry. NIR spectroscopy is a rapid, non-destructive technique and requires none or minimal sample pretreatment.

The NIR region spans the wavelength range 12,500–4000 cm^{-1}. In this region, absorption bands correspond mainly to overtones and combinations of fundamental vibrations [2].

In the pharmaceutical sector, several qualitative and quantitative applications of NIR spectroscopy have been described during manufacturing steps.

In the beginning of manufacturing process, NIR can be used for the identification of active substances and excipients [3–5]. By recording a NIR spectrum, it has been shown that identity, crystallinity, and water content are controlled

* Corresponding author. Tel.: +32 4366 43 01; fax: +32 4366 43 02.
E-mail address: cbodson@ulg.ac.be (C. Bodson).

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making NIRS an interesting tool for the characterisation of raw materials.

The blending step can also be followed by NIRS [6]. It is well known that creating homogeneous powder blend is one of the most important step during manufacturing of solid dosage forms in pharmaceutical industries. Typically, the most time-consuming part of the blending process is not the blending itself but the analysis that must be performed to validate the final homogeneity of the drug substance in the blend. Usually, to test the homogeneity of the blend, manufacturers remove some samples which are representative of the powder bed with a thief probe. The samples are then analysed by traditional methods such as UV–vis spectrophotometry or high performance chromatography.

NIR spectrophotometry can also be used for the assay of pharmaceutical dosage forms. With advances in instrumentation and software, rapid characterisation of intact dosage forms has become a reality. As far as quantitative analysis is concerned, NIR spectroscopy allows the determination of active substances in various pharmaceutical preparations. More recently, the assay of active drugs in tablets has been reported. This assay can be performed by reflectance [7] or transmittance [8] but it was shown that the use of transmittance measurements has brought the advantage over reflectance NIR spectroscopy because these measurements are less sensitive to the heterogeneity of the material, which is a problem in reflectance analysis [9]. In their paper, Gottfries et al. have shown that models based on transmission spectra give a better prediction power than models based on diffuse reflectance spectra.

In practice the relationship between concentration and absorbance is empirically determined by calibration. In the first step, spectra of substances with known composition are recorded. Then, these acquired spectra and the data available from a reference analysis are used to determine a calibration function. In the second step, spectra of substances with unknown composition are measured and then used to determine the properties of interest by means of the calibration function [10,11].

Processing NIR data can be carried out in a number of ways to simplify the spectral information. It has been shown that data pretreatment could be a key step for success of NIR spectroscopy [12].

In this article, a NIR technique is described to monitor the blending process and to assay low-dosage tablets containing riboflavin. The aim of the study is to show the agreement between the NIR technique and a conventional UV–vis spectrophotometry assay method.

2. Experimental

2.1. Materials and methods

2.1.1. Materials

Microcrystalline cellulose (Avicel® PH 200 FMC, Belgium) was used as diluent and binding agent. Riboflavin (Roche®, Germany) was chosen as a model drug substance.

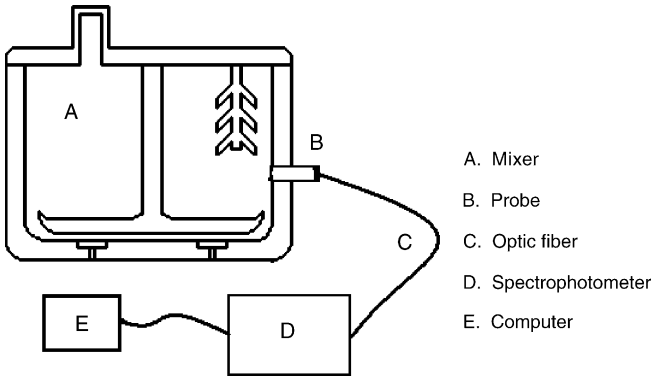

Fig. 1. NIR equipment for the powder blend on-line analysis.

2.1.1.1. Mixing homogeneity. The mixing was performed with a Gral 10 high shear mixer (GEA-Collette nv, Wommelgem, Belgium). The process bowl has a volume of 10 l. For in-line monitoring, the jacketed bowl was perforated to allow the introduction of a NIR fibre probe. Fig. 1 shows the instrumentation of the bowl. The mixing process was monitored for 30 min with the main impeller rotating at 400 rpm. The blend load was 1.5 kg.

Sampling: To confirm the end point of mixing obtained by NIR, a sampling technique was performed with a single compartment end-sampling thief probe. Ten samples were taken from the powder blend in the bowl. Samples were considered uniform if the drug concentration of each individual sample was within 10% of the average concentration and the relative standard deviation (R.S.D.) was less than 5%.

2.1.1.2. Tablets assay. Tablets were manufactured with a rotary press RO/2 from GEA-Courtoy (Belgium) using flat 7 mm diameter punches.

2.1.2. Analytical procedures

2.1.2.1. NIR spectroscopy. Equipment and software: The spectrometer used was a multi-purpose analyser (MPA) from Bruker Optics (Belgium). This spectrometer was designed for the routine lab as well as for at-line applications and method development.

The fibre optic module and the transmission unit were used in this application. The fibre optic module was used to follow the mixing kinetics and the transmission unit was used for the tablet assay.

The reflectance probe was a Series 400 Diffuse Reflectance Probe from Precision Sensing Devices (Massachusetts, USA). It was linked to the spectrometer with a bifurcated fibre optic from Precision Sensing Devices.

The detector used by both reflection and transmission measurements is a high sensitivity InGaAs detector.

The MPA was completely software controlled by the OPUS software Version 5.0 which was provided by Bruker Optics. OPUS IDENT is a software package designed to identify substances by their NIR spectra while OPUS Quant is designed for the quantitative analysis.

For this purpose, QUANT used a partial least square (PLS) fit method. In PLS, the calibration involves correlating the data in the spectral matrix X with the data in the concentration matrix Y.

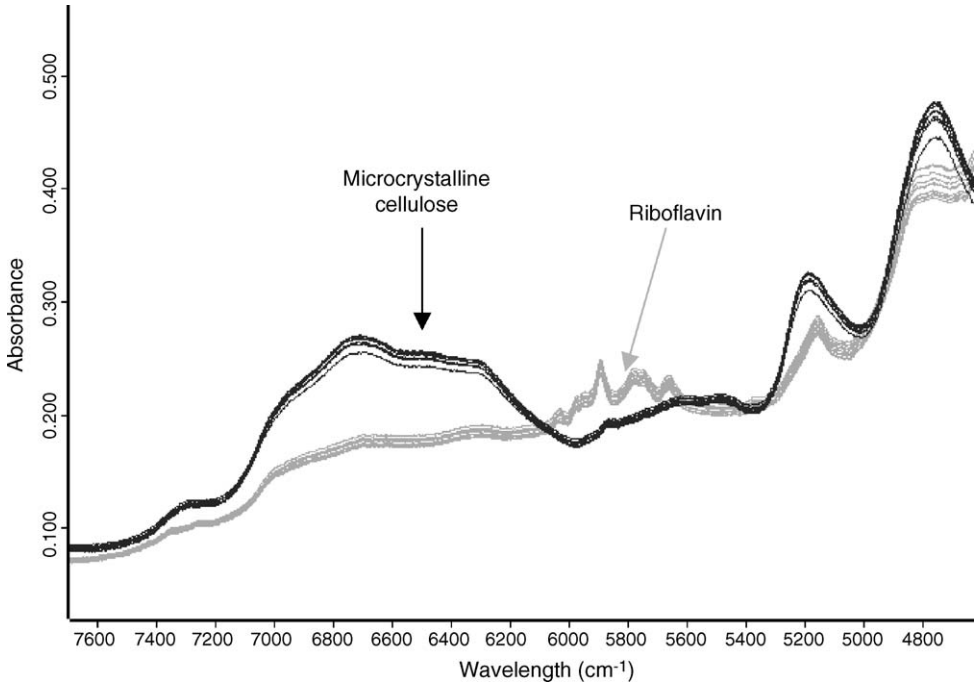

Fig. 2. NIR raw spectra of riboflavin and microcrystalline cellulose.

The *X* and *Y* matrices are reduced to only a few factors using all of the available information. The final model consists of a score matrix for *X* and a score vector for *Y* which are linearly related. This means that the factoring of the spectral data is more suited for concentration prediction.

Construction of the PLS model: In a first step a PLS regression model was built using calibration samples. The obtained model was chemometrically validated by leave-one-out cross-validation. The final PLS model was described by a selected spectral region, a certain spectra pretreatment and a number of PLS factors. To build the model, five different concentration tablets were prepared and ten tablets were measured per concentration.

To obtain these different concentrations, only the tablet weight was varied during the production on the rotary press. The tablet weights were 80, 100, 120, 140, and 160 mg with each tablet containing the same active substance centesimal composition. Each spectrum was the average of 32 scans and the spectrophotometer was operated at a resolution of 8 cm^{-1}.

Spectral data pretreatments: NIR spectra are affected by the state of the analysed material (i.e. powder surface, tablet surface, etc.). The baseline can drift and maximum absorbance may change. Spectral pretreatments correct these interferences [13,14]. In our study, a normalisation and a first derivative were used to enhance spectral information and to reduce baseline drift. The normalisation method used was a vector normalisation.

This method calculates the average *y*-value of the spectrum. The average value is subtracted from the spectrum decreasing the mid-spectrum to $y = 0$. The sum of the squares of all *y*-values is calculated and the spectrum is divided by the square root of this sum.

After the normalisation, a first derivative was applied using the Savitzky-Golay algorithm. This algorithm allows a simultaneous smoothing of the spectrum to reduce the noise generated by derivation. The number of smoothing points was 17.

UV–vis reference method: The reference method was a UV–vis cell measurement of absorbance at 440 nm, path length 10 mm, using a HITACHI U-3010 spectrometer (Tokyo, Japan). As it is a destructive method, it was performed on powder sample or tablet one by one after the NIR measurement.

Riboflavin from each powder sample or tablet was dissolved in 25 ml of a 14 g/l anhydrous sodium acetate solution. Each solution was stirred for 1 h 30 min and then centrifuged at 4000 rpm for 15 min. A 1-ml volume of the supernatant was diluted to 20 ml with the sodium acetate solution.

2.1.3. Agreement between the two methods for unknown samples: validation of the model

Fifty unknown samples were randomly taken at different concentration levels. All these tablets were assayed by NIR spectroscopy using the building model for 3 days. The third day, these tablets were assayed by UV–vis spectrophotometry.

To compare the two techniques a statistical method described by Bland and Altman was used [15].

3. Results and discussions

3.1. Powder mix homogeneity

3.1.1. NIR spectroscopy

The conformity test was used to follow the mixing kinetics [16]. The conformity test is an easy method to test the deviation of measured NIR spectra within certain limits. To set these limits, samples of the final product are needed as reference spectra which belong to at least one batch or one production cycle. These reference spectra vary within the accepted range

of specifications. The NIR spectra of these samples reflect the different sample variations and give a confidence band in the spectral range. To pass the conformity test, the spectrum of a new sample has to be within this confidence band at each wavelength.

First, the average and the standard deviation σ of the absorbance values have to be calculated for each wavelength. The mean value $\pm$ the standard deviation determines the confidence band within the spectral range and defines which amount of variations on each spectral wavelength is acceptable for the particular product.

Secondly, it has to be checked whether the spectrum of a sample to be tested is within the defined confidence band in the spectral range. The difference between this sample and the average of the reference samples is calculated on each wavelength i. This absolute deviation is weighted by the corresponding standard deviation σ at the respective wavelength, which results in a relative deviation referred to as conformity index (CI).

$$\mathrm{CI} = \frac{A_{\mathrm{reference},i} - A_{\mathrm{sample},i}}{\sigma_{\mathrm{reference},i}}$$

The first step of our study was to take the NIR reflectance spectra of the pure components. A range with a weak noise to signal ratio was selected (7500–4600 cm^{-1}). These spectra are shown in Fig. 2 whereas Fig. 3 shows the first derivative of these spectra.

In Fig. 4, a decrease of the conformity index is observed during the mixing. The homogeneity is reached when the conformity index remains constant.

3.1.2. Sample thief technique

The mixing homogeneity was confirmed by a validated UV–vis spectrophotometry method.

Ten samples were taken and assayed after each blend. Fig. 5 shows that each sample was within 10% of the mean concentration and that the relative standard deviation value was less than 5%.

3.2. Tablet assay

Fig. 6 shows the raw spectra obtained with the calibration samples. A vector normalisation was applied to these spectra.

From these spectra, two regions were selected, the first one between 11,250 and 8500 cm^{-1} (see Fig. 7), the second one between 7900 and 7350 cm^{-1} (see Fig. 8).

All tablets were also analysed by a validated UV–vis spectrophotometry method.

The linearity, the trueness, and the precision (repeatability and intermediate precision) were assessed.

3.2.1. Linearity

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of the analyte in the sample [17,18].

A good linearity was obtained. The regression model gives the following equation:

$$Y = -0.00373 + 0.995X$$

where Y are the results (μg) and X is the introduced amount (μg).

The coefficient of determination (r^2) is equal to 0.997.

3.2.2. Trueness

The trueness of this validated UV–vis technique is presented in Table 1. The trueness refers to the closeness of agreement between a conventionally accepted value or reference value and

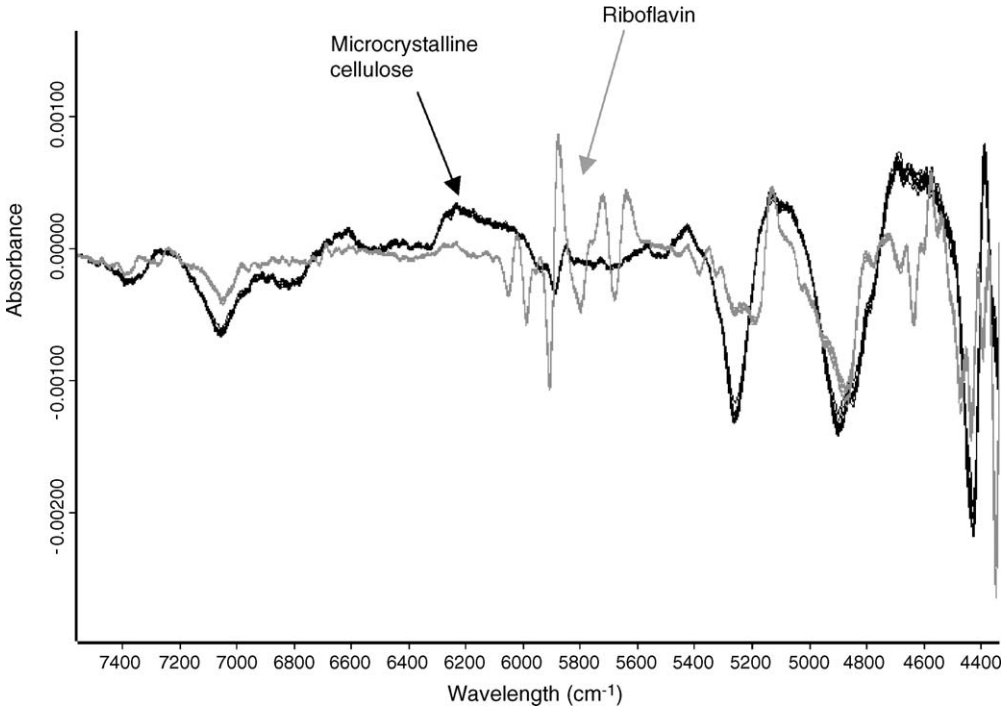

Fig. 3. First derivative spectra of microcrystalline cellulose and riboflavin.

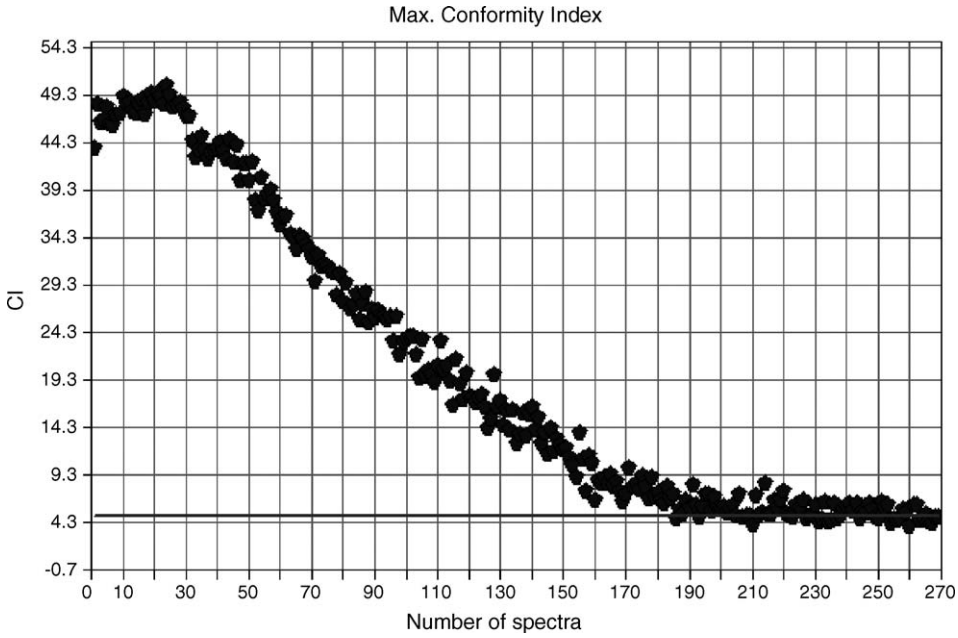

Fig. 4. Evolution of conformity index during mixing.

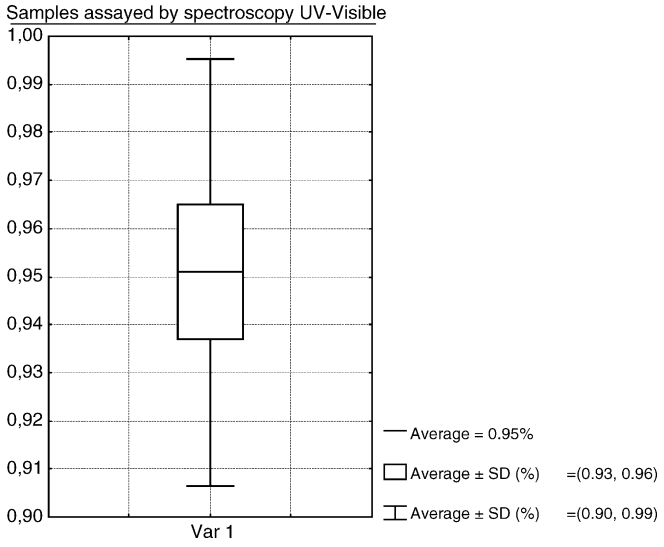

Fig. 5. Assay of powder blend samples by UV–vis spectrophotometry.

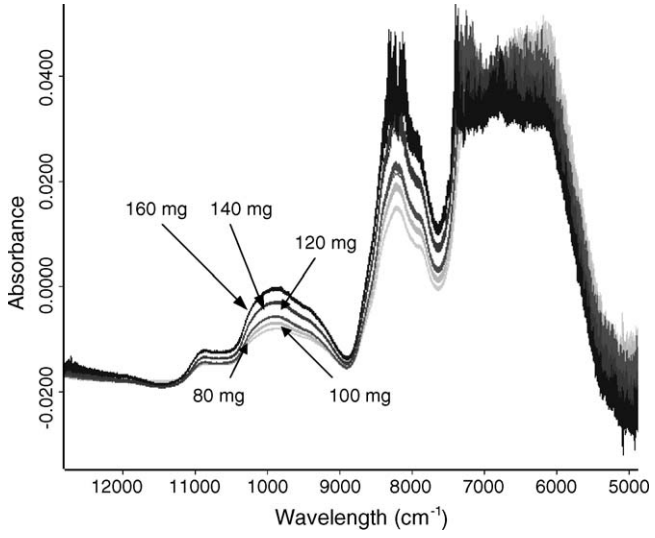

Fig. 6. NIR raw spectra of calibration tablet after vector normalisation.

Table 1
Trueness of the UV–vis tablet assay

Amount level	Absolute bias (μg)	Relative bias (%)	Recovery (%)
1	-1.67×10^{-3}	-6.59×10^{-2}	99.93
2	7.22×10^{-3}	0.15	100.1
3	-3.17×10^{-2}	−0.42	99.58
4	-1.44×10^{-2}	−0.15	99.85
5	5.01×10^{-3}	4.12×10^{-2}	100.0

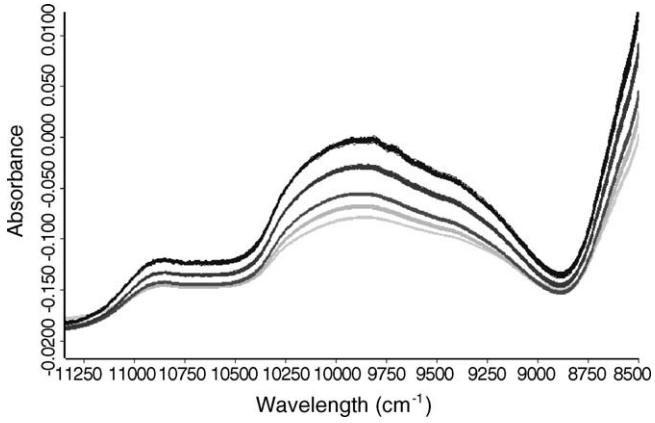

Fig. 7. Range between 11,250 and 8500 cm^{-1}.

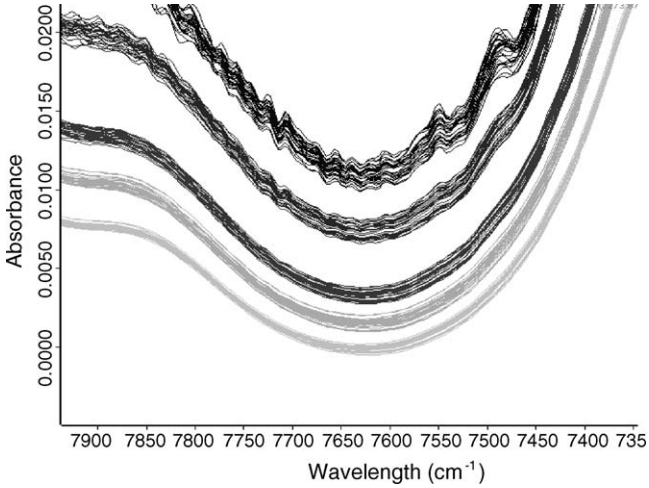

Fig. 8. Range between 7900 and 7350 cm^{-1}.

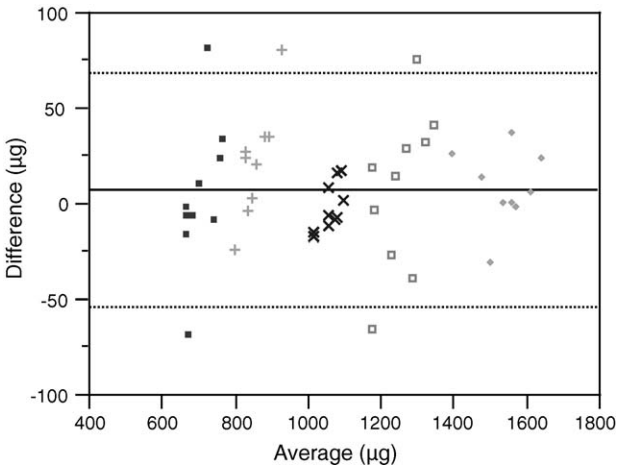

Fig. 10. Distribution of the differences between the two methods against their mean.

Table 2
Precision of the UV–vis tablet assay

Amount level	Repeatability (R.S.D.%)	Intermediate precision (R.S.D.%)
1	2.5	2.8
2	1.2	1.5
3	0.7	0.8
4	0.4	0.4
5	0.3	0.4

a mean experimental one [18]. It gives information on systematic error.

3.2.3. Precision

The precision of this validated UV–vis technique is presented in Table 2. Precision is the closeness of agreement among measurements from multiple sampling of a homogeneous sample under the recommended conditions [19]. It gives some information on random errors and it can be evaluated at two levels: repeatability and intermediate precision.

To evaluate the predictability of the model, the relative standard error of prediction (RSEP) was used [19]:

$$\mathrm{RSEP}\,(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_{\mathrm{UV-vis}} - C_{\mathrm{NIR}})}{\sum_{i=1}^{n} C_{\mathrm{UV}}}}$$

where C is the amount of riboflavin as measured by the UV–vis (reference) and the NIR method and n is the number of samples.

The chosen model had a RSEP value of 2.7%. This regression model gave a coefficient of correlation (r^2) of 99.23. This regression is shown in Fig. 9.

3.3. Agreement between the two methods for unknown samples: validation of the model

According to Bland and Altman's method, the first step is to examine the data. A simple plot of the results given by a

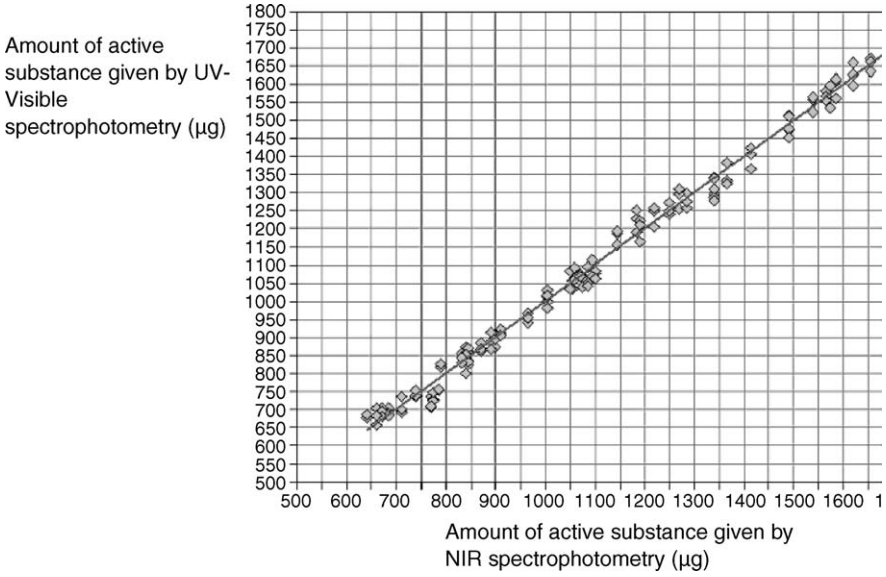

Fig. 9. Regression of the calibration samples.

Table 3
R.S.D. (%) of the amount of riboflavin obtained by the two techniques at each concentration level

	Concentration level				
	1	2	3	4	5
NIR	4.15	2.35	4.20	4.56	4.76
UV–vis	5.92	3.13	5.99	7.50	4.76

method versus those of the other one is a useful start. However, the data points will usually be clustered near the line and it will be difficult to assess between method differences so that a plot of the difference between the methods against their mean is chosen. This plot of data may be more informative. The lack of agreement could be summarised by calculating the bias, estimated by the mean difference $\bar{d}$ and the standard deviation of the differences (S.D.). Fig. 10 shows the distribution of the differences against their mean.

Five points are outside of the limit $\bar{d} \pm 2$ S.D. This could be explained by a more important variability of the UV–vis spectrophotometry method compared to the NIR technique. The higher variability of the UV–vis technique could also be observed by the R.S.D. values (%) at each concentration level. Table 3 shows these R.S.D. values (%).

For each level concentration (except the last one), R.S.D. of the UV–vis absorbance values is always higher than R.S.D. of the NIR spectroscopy measurements.

The fact that five points are outside the limit ± 2 S.D. is not a problem because these points represent the difference between the two methods; moreover, in the range of interest (1000 μg), there is no problem.

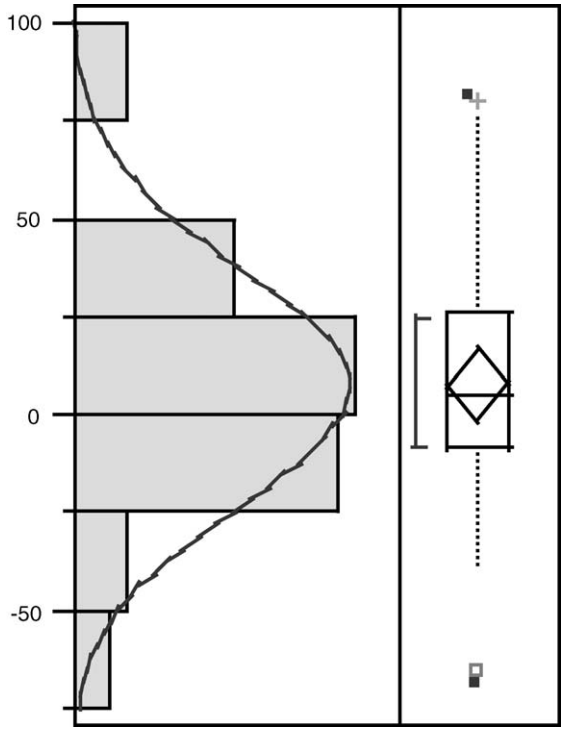

Fig. 11. Normal distribution of differences.

Table 4
Trueness of the NIR tablet assay

Amount level	Absolute bias (μg)	Relative bias (%)	Recovery (%)
1	8.0×10^{-2}	1.11×10^{-2}	100.0
2	−0.4786	-5.66×10^{-2}	99.94
3	0.86	8.03×10^{-2}	100.1
4	-0.27×10^{-2}	-2.12×10^{-2}	99.98
5	−5.00	−0.32	99.68

Table 5
Precision of the NIR tablet assay

Amount level	Repeatability (R.S.D.%)	Intermediate precision (R.S.D.%)
1	3.72	3.86
2	4.29	4.30
3	2.30	2.44
4	4.26	2.26
5	4.45	4.45

Afterwards, the distribution of differences has been observed (Fig. 11). This distribution is normal with a mean of 7.14 μg and a standard deviation of 30.46 μg.

After 3 days of measurement, the trueness, the precision, and the accuracy of the NIR method were evaluated as shown in Tables 4 and 5 and in Fig. 12.

Accuracy refers to the closeness of agreement between the test results and the accepted reference values. The accuracy takes into account the total error, i.e. systematic and random errors, related to the test result. The acceptance limits were set at ±15% as described in the European Pharmacopoeia for tablet containing less than 2 mg of active substance.

Fig. 12 shows the accuracy profile of the NIR technique.

The plain line is the relative bias, the dashed lines are the β-expectation tolerance limits and the dotted curves represent the acceptance limit (15%) [18,20,21]. We can see on this graph that there is no relative bias in measurements when the predicted model is used.

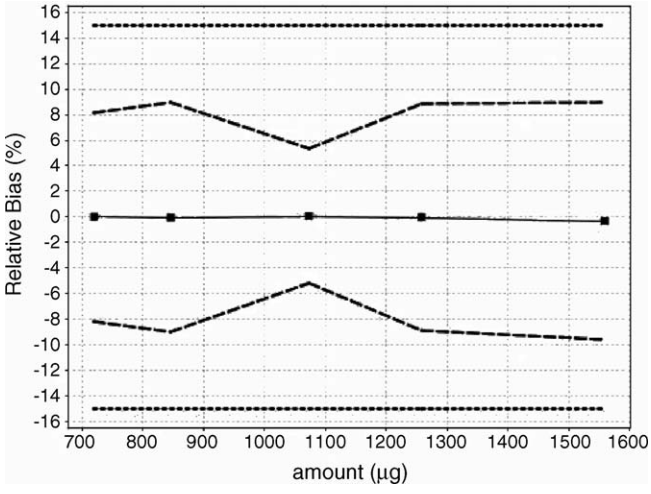

Fig. 12. Accuracy profile of the NIR technique.

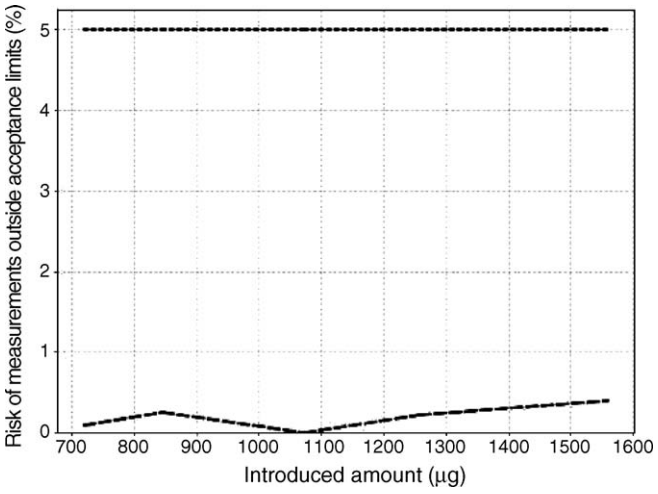

Fig. 13. Risk profile of the NIR technique.

This approach gives the guarantee that each further measurement of unknown samples is within the tolerance limits at the 5.0% level.

The risk profile of our method was evaluated and for all the range, the risk is less than 1%.

Fig. 13 shows the risk profile, the dotted line represents the maximum risk level chosen (5%).

4. Conclusions

It is most unlikely that different assay methods will agree by giving the identical result for all individual assays. In this study, we want to show how a new method differs from a conventional one. The results obtained in this study show that the NIR spectroscopy technique offers some advantages over the conventional UV–vis spectrophotometry method. Compared to the conventional technique, the NIR spectroscopy method is faster, non-destructive, and gives less variability. It has been shown that NIR spectroscopy can replace safely the UV–vis spectrophotometry.

Acknowledgements

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