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# Identification and quantitation assays for intact tablets of two related pharmaceutical preparations by reflectance near-infrared spectroscopy: validation of the procedure

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**Abstract**

Quantitative analysis based on near-infrared (NIR) spectroscopy uses individual calibration equations for each sample studied because of the need to model all possible chemical and physical variability sources. A NIR method, using a fibre optical probe, for the analysis of two different and related pharmaceuticals from two different production steps (cores and tablets) is studied. Both pharmaceuticals have the same active principle and similar excipients, but with different nominal concentration values. The pharmaceuticals are identified by comparison using a second-derivative spectral library; an identification procedure consisting of two-steps (cascade) library: correlation coefficient followed by maximum distance in wavelength space is proposed. Once a sample has been positively identified, the active principle is quantified with partial least-squares regression (PLSR) using a sole and global calibration. The proposed method was validated for use as a control method, and for this purpose selectivity of the identification process, and the repeatability, intermediate precision, accuracy, linearity, and robustness of the active principle quantitation, were assessed. 

*Keywords:* NIR; Pharmaceutical preparations; Intact tablets; Validation

## 1. Introduction

Quality control in the pharmaceutical industry involves analyses of raw materials, products prior to dosing and end products, which entails a large number of analyses for each preparation. A proper control process has to meet the following criteria: should be sufficiently accurate and precise; the sample pretreatment required should be none or minimal; should allow several analytes to be simultaneously determined and should enable expeditious control of the manufacturing process. Near-infrared (NIR) spectroscopy is among those analytical techniques that most closely meets the requisites [1,2].

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NIR absorption is mainly due to overtone and combination vibrations arising from fundamental vibrations and also vibrations of crystals in the mid-infrared region. As these absorptions are comparatively weak, NIR spectroscopy should be ideally suited for the analysis of major components. Analysis based on NIR spectroscopy uses independent calibration equations for each type of sample because of the need of modelling all possible chemical and physical variability sources.

One of the best advantages of NIR spectroscopy is the possibility of working in reflectance and the use of suitable cuvettes or a fibre optical probe module coupled to the spectrophotometer to make measurements with no sample preparation, thereby avoiding the need of reagents and solvents. Although the most common use of fibre probes in routine analyses is the identification of raw materials, its potential for quantitative analyses has also been demonstrated [1–7]. As regards qualitative applications, NIR spectroscopy has solved various problems such as preliminary investigations in the analysis of mixtures or discrimination among similar products; more widespread, however, is the identification of pure chemicals by reference to an existing spectral library. In this work, we assayed different identification methods to identify the spectrum for the unknown sample with one in the library and discriminate two related pharmaceuticals from each other and from its pure compounds.

As far as quantitative analysis is concerned, NIR spectroscopy allows the determination of active principles and/or excipients in various pharmaceutical preparations by using different multivariate calibration techniques, of which partial least-squares regression (PLSR) is the most widely employed choice. In quantitative analysis changes on the spectra due to physical properties are minimised by applying spectral pretreatments and/or including variability in the calibration to model it. A problem with intact tablet assays is that the normal production batches do not encompass a sufficiently wide range for setting up a reliable calibration equation. The expansion of the range for calibration can be done in two models. One is making under- and overdosed tablet samples from a pilot plant [8]. The other using powdered samples, which could be done in two ways. Under- or overdosing powdered production samples [9], or making powdered laboratory samples containing all the pharmaceutical components in concentrations within the manufacturer's accepted range, which simplifies significantly costs and preparation time. This methodology could give some errors if the procedure is not strictly identical to that used by the manufacturer, however, we have used this method previously with highly satisfactory results [5,6,9].

Pharmaceuticals in tablet form usually belong to two production steps: cores (the compressed pharmaceutical with an specific shape) and coated tablets (final product). It is also desirable that the constructed calibration could work successfully with both production steps. Moreover, in case of related pharmaceuticals to simplify the method a unique calibration should work for all of them.

Once a new analytical method has been developed, it must be validated if it is to be accepted for use in routine analyses; in this way, the method is guaranteed to perform in such a way as to provide quality results every time.

Recently, we described the application of NIR spectroscopy to the analysis of intact tablets for two related pharmaceuticals [5,6] (same active principle and similar excipients with different composition), but we have studied both separately (with fibre optic probe and RCA module, respectively), using different calibrations. This paper describes a simultaneous and rapid identification and quantitation assay for the determination of gemfibrozil in two final production steps, cores and coated tablets, of two related pharmaceuticals using NIR spectroscopy. Where possible we applied the recommendations of the International Conference of Harmonisation (ICH) guidelines [10] to validate the assay. The ICH guidelines recommend the study of linearity, range, accuracy and precision, both short and long term and robustness, for analytical procedures, which are to be used for pharmaceutical registration purposes.

## 2. Experimental

### 2.1. Samples

The samples studied were two commercially available pharmaceuticals, Trialmin 600 (T600) and Trialmin 900 (T900) from Laboratorios Menarini (Badalona, Spain), available as coated oval tablets. Two production steps were studied: cores (T600C and T900C) and coated tablets (T600T and T900T). Table 1 lists the concentration values for each component by both pharmaceuticals. As can be seen both pharmaceuticals have the same active principle, gemfibrozil (viz. 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid) but with different nominal levels, 751 mg/g in T600C and 810 mg/g in T900C.

In all, 32 T600 samples (18 cores and 14 coated tablets) and 35 T900 samples (23 cores and 12 coated tablets) were used. The active principle content in each production sample was obtained as the average of two determinations by the UV reference method.

Moreover, 27 powdered samples containing various amounts of the active compound and the excipients at gemfibrozil concentrations within 631–916 mg/g were prepared in the laboratory by weighting the individual components. These were thoroughly mixed in a shaker mixer before recording their NIR spectra. The active principle concentration used as a reference was the weighting value.

Table 1
Nominal concentration values for each component by the studied pharmaceuticals in core presentation

| | Trialmin 600 (mg/g) | Trialmin 900 (mg/g) |
|---|---|---|
| Gemfibrozil[a] | 751 | 810 |
| Pregelatinated starch | 130 | 136 |
| Colloidal silica | 17 | 17 |
| Carboximethyl starch | 13 | 12 |
| Magnesium estearate | 6 | 6 |
| Cellulose microcristalline | 75 | – |
| Polisorbat | 8 | 7 |
| Coated[b] | ~30 | ~30 |

[a] Active principle.
[b] Added amount to total cores weight.

Samples of the pure components and of the pharmaceutical preparations from different production batches were supplied by Laboratorios Menarini.

### 2.2. Apparatus

Near-infrared spectra were recorded on a NIRSystems 5000 spectrophotometer from Perstorp Analytical (NIRSystems, Maryland, USA). The instrument was equipped with a P6645 ANO3P fibre-optic module for quantitative analyses. The system was governed by the NSAS v. 3.52 software suite, also from NIRSystems, for acquisition and processing of spectra.

The UV spectra used in the reference method were recorded on a Hewlett-Packard (Waldbronn, Germany) HP8452A diode array spectrophotometer. The instrument's bundled software HP 89530 MS-DOS UV/Vis includes facilities for controlling, acquiring and processing spectra. Absorbance spectra were recorded by using quartz cells of 1-cm path length.

The experimental set-up also included a Turbula T2C shaker mixer from WAB (Basle, Switzerland) used to homogenise laboratory-made solid samples, a Turmix Mill blade grinder (Barcelona, Spain), a Selecta ultrasonic bath (Abrera, Spain) and an Alresa centrifuge (Madrid, Spain).

### 2.3. Software

The multicomponent analysis software MC94, developed by the Unidad de Química Analítica in the Universidad Autónoma de Barcelona, was used in the UV analysis. It allows the concentrations of the components of a mixture to be determined by classical least-squares regression (CLS) fitting the mixed spectrum from the spectra for the pure components.

Correlation and maximum distance in wavelength space, and residual variance in principal component space implemented in VISION v.2.20

(FOSS, Didcot, UK) were used as identification methods.

Multivariate calibration was performed with UNSCRAMBLER v. 6.1 (CAMO, Trondheim, Norway), which affords PCA and PLSR, with selection of variables and samples and detection of outliers.

### 2.4. UV reference procedure

The following UV spectrophotometric procedure was used as a reference in the determination of the active compound (gemfibrozil) in production samples. About 0.2 g of milled sample was dissolved in 80 ml of methanol and the mixture was sonicated for 15 min and then diluted to 100 ml with the same solvent. An aliquot of the resulting solution was centrifuged at 3000 rpm for 5 min. A 3-ml volume of the supernatant was added to 2 ml of 0.1 M HCl and diluted to 50 ml with methanol. Finally, the spectrum for this last solution was recorded between 190 and 350 nm against a blank consisting of 2 ml of 0.1 M HCl diluted to 50 ml with methanol.

The program MC 94 was used to quantify the samples from their first-derivative spectrum over wavelength range 250–290 nm, using the first-derivative spectrum of the active compound as the sole standard.

### 2.5. Recording of NIR spectra

The spectrum for each sample was recorded in triplicate over the wavelength range 1100–2498 nm, from an average of 32 scans. Spectra were recorded in the reflectance mode, using a fibre-optic module. Laboratory samples spectra were obtained by direct insertion of the fibre-optic probe into the same wide mouth plastic bottle in which the sample was weighted and mixed. The sample was turned over with a spatula between consecutive measurements (three in all). The spectra of the tablets were recorded in a custom-built holder consisting of a black plastic tube that was mechanised to fit the tip of the probe [6]. The tube was partially filled with several tablets and the probe was brought into direct contact with the sample; after each run, the tube was shaken to change the position of the tablets within. Fig. 1 shows the NIR spectra for the different pharmaceuticals and pure components.

#### 2.5.1. Data processing

The proposed analytical procedure uses a single spectral measurement for the simultaneous qualitative and quantitative analysis of two related commercially available pharmaceutical preparations.

All libraries and quantitation models tested were constructed from second-derivative spectra in order to facilitate discrimination among different products and minimise spectral variability due to scattering, and encompassing 1100–1450, 1600–2200 wavelength ranges, and thus avoided Wood's anomalies [11] and the high background noise above 2200 nm associated with the use of a fibre optical probe.

#### 2.5.2. Identification (ID)

Three ID methods were assayed: correlation and maximum distance in wavelength space and residual variance in principal component space. Samples were identified by comparison of their spectra with standard spectra in a reference library. To this end, a library containing spectra for the pharmaceutical preparations, the active principle and the excipients was compiled; for each product, a set of sample spectra that met the specifications and represented all the variability in the manufacturing process were available.

The correlation coefficient [12,13], widely used for expressing similarity, is defined as the cosine of the angle between the vectors for the sample spectrum and the average spectrum for each product included in the library:

$$\rho_{jk} = \frac{\sum_{i=1}^{p} (x_{ij} - \bar{x}_j)(x_{ik} - \bar{x}_k)}{\sqrt{\sum_{i=1}^{p} (x_{ij} - \bar{x}_j)^2} \sqrt{\sum_{i=1}^{p} (x_{ik} - \bar{x}_k)^2}}$$

where $p$ is the number of wavelengths; subscripts k and j denote the sample and reference product, respectively; $x_i$ is the measured value at wavelength $i$; $\bar{x}_j$ is the average spectrum for reference product $j$; and $\bar{x}_k$ is the average spectrum for the sample.

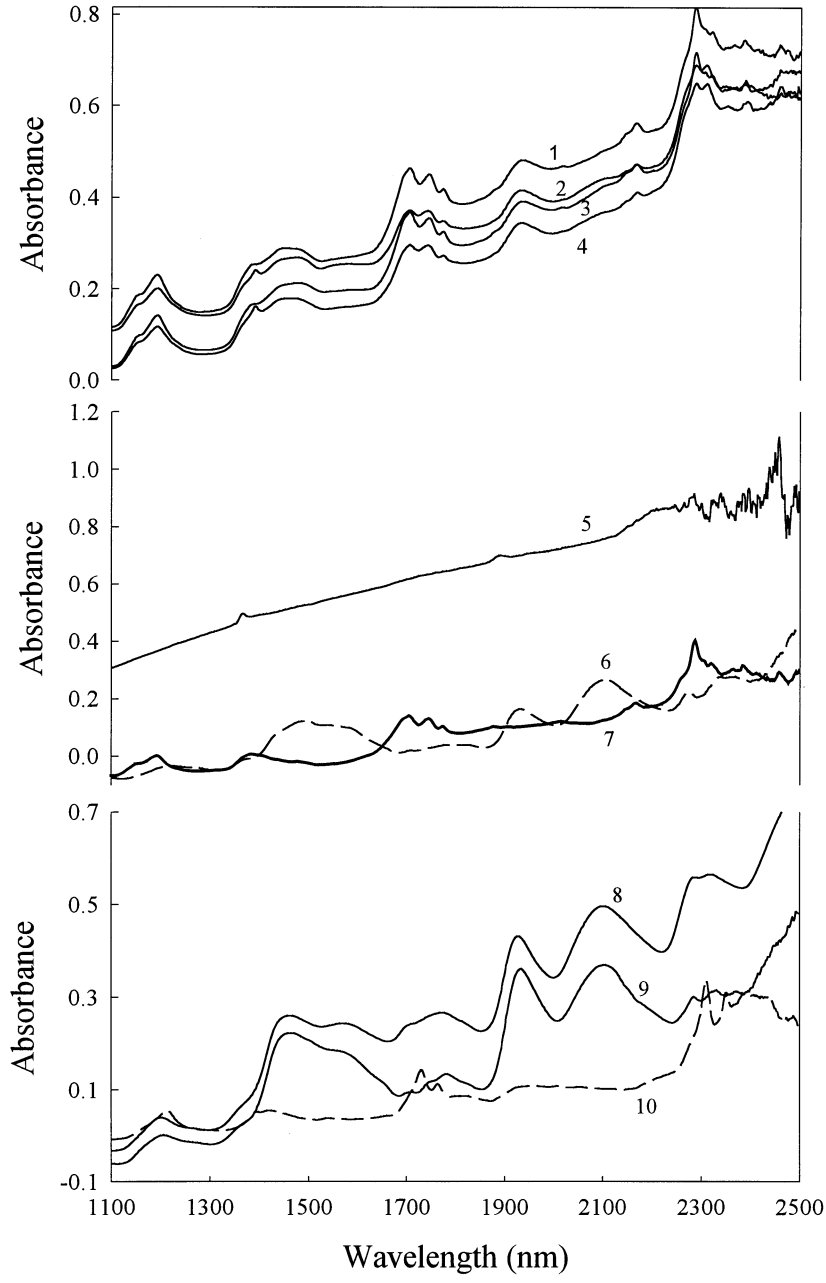


Fig. 1. NIR spectra for (1) T900C, (2) T900T, (3) T600C, (4) T600T, (5) colloidal silica, (6) cellulose microcristalline, (7) gemfibrozil, (8) carboximethyl starch, (9) pregelatinated starch and (10) magnesium stearate.

Theoretically, if the two spectra are coincident, the correlation coefficient should be unity; however, random noise associated to any type of spectral measurement precludes obtaining a coefficient of exactly 1. If the similarity coefficient exceeds a preset threshold, then the two spectra compared are considered identical and the sample is identified with the reference product.

Wavelength distance method [13,14] assumes that measurements at each wavelength are distributed according to the normal law. It generally uses the second derivative of spectra from a library that defines the accepted variability for the product to obtain an average spectrum and the standard deviation at each wavelength. The distance between the unknown sample and the average spectrum for the reference product at each wavelength is calculated and the most unfavourable situation (viz. the wavelength that results in the maximum distance) is determined from the following formula:

$$d_{\mathrm{kj}} = \max \frac{|x_{\mathrm{k}p} - \bar{x}_{\mathrm{j}p}|}{s_{ij}}$$

where subscripts k and j denote sample and reference product, respectively; $x_{\mathrm{kp}}$ is the measured sample value at wavelength $p$; $\bar{x}_{jp}$is the average spectrum for reference product j at wavelength $p$; and $s_{\mathrm{jp}}$ is the standard deviation of the measured values for reference product j at wavelength $p$.

The most commonly used qualification criterion is based on the expression $d_{\max} \leq 3$, but it could be too conservative; it is often more practical to have users decide upon the most suitable limit for their own problems and working methods.

One shortcoming of this method is the risk of false negatives at wavelengths coinciding with $x$-intercepts in second-order derivative spectra (zero crossover). If the standard deviation for the average spectrum at a given wavelength is very small, then the distance at that wavelength will be very large and a negative qualification will result. This may be the case when second-derivative values are very close to zero. This problem can be circumvented by using the wavelength library stabilisation method [15].

The other classification procedure is the residual variance method [16]. Each of the products in the sample is subjected to PCA and Fisher's test is subsequently applied in order to estimate the likelihood of a sample belonging to the class defined by the spectra for the reference product. The residual variance for a spectrum $k$ to be identified ($S$) that is assumed to belong to class j (defined by the spectra for reference product j) is divided into the total variance for the samples belonging to class j ($S$) in order to obtain the following variance relation:

$$F = \frac{S_{\mathrm{k}}^2}{S_0^2} \frac{n}{n-a-1}$$

where $n$ is the number of spectra for the reference product and $a$ that of PCs used to construct the class model. From the $F$ function one can calculate the probability that a given sample belongs to the distribution represented by the training set.

### 2.5.3. Quantitative analysis

All models tested were based on the PLS algorithm [17] and constructed by cross-validation, using as many segments as samples in the calibration set. The number of significant PLS components was taken to be the minimum number for which the prediction error sum of squares (PRESS) was not significantly different from the lowest PRESS value [18].

The quality of the results was assessed in terms of the relative standard error of prediction (RSEP(%)) [19]:

$$\mathrm{RSEP}(\%) = \sqrt{\frac{\Sigma_{i=1}^{n}(C_{\mathrm{NIR}_i} - C_{\mathrm{LAB}_i})^2}{\Sigma_{i=1}^{n} C_{\mathrm{LAB}_i} 2}} \times 100$$

where $n$ is the number of samples, $C_{\mathrm{LAB}}$ the reference concentration and $C_{\mathrm{NIR}}$ the PLS calculated concentration.

## 3. Results and discussion

### 3.1. Development and validation of the ID method

A library consisting of 258 spectra for ten different products, viz. two production steps for both pharmaceutical preparations, and its six pure components were compiled using the correlation method. An unknown sample was assumed to be positively identified if its correlation coefficient exceeded the established threshold ($\rho = 0.85$); if any sample surpassed such a threshold for more than one product in the library, it was positively matched to that with the higher coefficient.

The self-validation of this library shows that

there are conflicts among similar products; the higher ambiguities are between T600C and T900C, and between T600T and T900T, due to the important spectral similarity between these products (Fig. 1), but there are also some mismatches between cores and tablets. This library allows us to discriminate between the pharmaceuticals and their pure components, but it doesn't work to identify separately each pharmaceutical from each production step.

A two-steps library is proposed. In the first step the correlation coefficient allows us to discriminate between pure components and pharmaceuticals, and in the second step another identification method is used to discriminate among the pharmaceuticals. As can be seen in Fig. 1, the pharmaceutical spectra for different products are very similar, so a method with a high discriminating power must be used in the second step.

Two ID procedures based on two-steps (cascade) were assayed: correlation coefficient + distance (library A) and correlation coefficient + residual variance (library B). The established thresholds for the new methods were $d_{max} \leq 4$ in distance method, and probability $< 0.84$ for the residual variance method; in both cases, if any sample surpassed such a threshold for more than one product in the library, it was positively matched to that with the smallest value.

Using any of both proposed libraries, the self-validation does not exhibit errors and all the spectra included in the library were correctly identified.

To validate the libraries 55 new samples (including different batches from each pharmaceutical from the two production steps) were analysed using the proposed ID methods (A and B). Both libraries identified correctly all the samples analysed, so any of them can be used as an ID library in the problem studied. In this work, the ID method based on correlation coefficient plus distance is proposed as the optimum, since the distance method usually needs less samples than the residual variance method to construct the library [20].

### 3.2. Development of the quantitation method

Available samples were split into a calibration set and a prediction set. The calibration set consisted of laboratory samples and samples from different batches of both pharmaceuticals and the two production steps. The powdered laboratory samples spans the whole concentration range of interest in a uniform manner, these samples do not have the same physical properties that the real samples have (tablets) and production samples, cores and tablets, were included in the calibration set in order to introduce the variability of the manufacturing process [4]. The real samples included in the calibration set were selected based on PCA; the samples selected are those that show the greatest variability in the plot of the first component against the second (in our case, first and second components accounted for 86% of the variance); this variability is basically due to the manufacturing process. After this selection the calibration set was composed of 41 samples, 14 of which were laboratory-made, 14 Trialmin 600 production batches (seven cores and seven tablets) and 13 Trialmin 900 production batches (seven cores and six tablets); the prediction was composed of 53 samples, 13 laboratory-made, 18 Trialmin 600 production batches (11 cores and seven tablets) and 22 Trialmin 900 production batches (16 cores and six tablets).

The best model needed five PLS components to determine the active compound. Table 2 gives the RSEP values observed in this model. The quantitation of the active compound resulted have a relative standard error of prediction for

Table 2
Relative standard error of prediction calculated for the calibration set (RSEPC) and prediction sets (RSEPP) of laboratory samples, T600C, T900C, T600T and T900T using the calibration model assessed

| | Laboratory samples | T600C | T900C | T600T | T900T |
|---|---|---|---|---|---|
| RSEPC (%) | 1.2 | 0.6 | 1.3 | 0.9 | 1.3 |
| RSEPP (%) | 1.5 | 1.3 | 0.7 | 1.4 | 1.2 |

Table 3
Validation parameters studied for the constructed calibration equation

| Parameter | Test | T600C | T900C | T600T | T900T |
|---|---|---|---|---|---|
| Repeatability | CV (%) | 0.6 | 0.6 | 0.5 | 0.6 |
| Intermediate precision | CV (%) | 0.6 | 0.5 | 0.8 | 0.8 |
| | ANOVA | N.S.E.[a] | N.S.E.[a] | N.S.E.[a] | N.S.E.[a] |
| Accuracy | *t*-test | $t_{exp} = 0.21$ | $t_{exp} = 1.39$ | $t_{exp} = 1.86$ | $t_{exp} = 1.41$ |
| | | $t_{tab} = 2.13$ | $t_{tab} = 2.13$ | $t_{tab} = 2.45$ | $t_{tab} = 2.57$ |
| Linearity | Linear regression | NIR value $= -2\ (\pm 54) + 1.00\ (\pm 0.07)$ reference value $(r = 0.988)$ | | | |
| Robustness | % Recovery | 99.9% ($s = 0.9$) | | | |

[a] N.S.E., no systematic errors.

laboratory samples below 1.5% and for production samples below 1.2%. This calibration model allows the accurate prediction of real-world samples with no adverse effect on the quantitation of laboratory samples.

The specification limit for acceptance of active principle in the pharmaceutical was $\pm 5\%$ of the nominal value, which is clearly larger than the prediction errors obtained with the optimum calibration procedure for the production samples. Therefore, the proposed calibration procedure is precise enough for use as a control methodology for both pharmaceuticals simultaneously.

### 3.3. Validation of the quantitation method

Once the method was established, the calibration equation was validated by considering repeatability, intermediate precision, accuracy, linearity and robustness. The concentration range spanned by the calibration is another parameter usually examined for calibration; however the use of PLS regression to determine the active principle entails the a priori selection of the concentration range of interest. During the validation process, solid evidence was obtained that the resulting quantitation errors were acceptable throughout the concentration range studied.

### 3.4. Repeatability

The repeatability was determined by measuring the gemfibrozil content of a single batch, one for each pharmaceutical and production step, six times within 1 day. Table 3 shows the results obtained from measurements made by the same operator on the same day. As can be seen, in all cases the coefficient of variation (CV) were less than 0.6%, which is well below the usual accepted limits ($< 1\%$).

### 3.5. Intermediate precision

The intermediate precision was assessed on a sample from each pharmaceutical and production step by evaluating two variable parameters in the routine control of the preparation, namely, day ($n = 3$) and operator ($n = 2$). Table 3 shows the CV values obtained which were within usual accepted limits for this type of test ($< 2\%$). The variability between days and that between operators were evaluated jointly by two-way analysis of variance (ANOVA), which revealed that neither source produced any systematic errors.

### 3.6. Accuracy

Because the results could be affected by physical properties of the samples, the accuracy of the proposed method was only evaluated in production batches. For this purpose the NIR results were compared with those provided by a well-characterised (reference) method.

A paired *t*-test was used to check whether the mean value and that held as true (viz. the average value provided by the UV method) were significantly different. Table 3 shows the results obtained for the prediction set, as can be deduced the results provided by NIR spectroscopy and the

reference method are not significantly different ($t_{exp} < t_{tab}$).

### 3.7. Linearity

Linearity is usually estimated by evaluating the goodness of the variation of the analytical signal as a function of the analyte concentration. With multivariate calibration, however, an alternative test suited to the methodology in question must be used. To determine the linearity of the proposed method, the NIR and reference results were compared via the following equation:

$$\text{NIR value} = a + b \times \text{reference value}$$

The samples used to check for linearity should span the whole concentration range studied, so production and laboratory samples were included in this study. Table 3 shows the results for 24 samples (13 laboratory and 11 tablets from different production steps and preparations), in the concentration range of 670–890 mg/g, used to assess linearity. As can be seen, the results provided by both techniques were quite consistent throughout the concentration range studied.

### 3.8. Robustness

The proposed NIR method involves no sample pretreatment, so the only experimental variables potentially affecting the results are those inherent in the spectrophotometer, which are set before any spectra are recorded.

The proposed analytical method can be validated by comparing its results with those of a reference method over a period of time. The production samples analysed in the accuracy study were manufactured and analysed over a period of 3 years. The results exhibit more than acceptable accuracy and coefficients of variation. Moreover, a new set of 18 samples from both pharmaceuticals, analysed during a period of 6 months after the method was validated, has shown a 99.9% recovery from the nominal value (Table 3). The method is thus reasonably robust.

### 3.9. Conclusions

A NIR method for two different but related pharmaceutical preparations in intact tablet form was developed. The NIR process had the advantages over the reference technique of requiring no sample preparation and being non-destructive.

The analytical process involves the identification of the unknown sample and the quantitation of the active principle in the pharmaceuticals using the same spectral data. Identification relies on a two-step method, correlation plus maximum distance in wavelength space methods. This procedure has proved enough discriminating power to the correct identification of the two related pharmaceuticals (each from two different production steps) from each other and its pure components.

After the sample is identified, PLS regression allows the quantitation of the active principle. The use of laboratory-powdered samples and production intact tablets from both pharmaceuticals in the calibration process allows the construction of a unique and common calibration equation, so the determination of gemfibrozil does not depend on the pharmaceutical studied. The method was validated following the recommendations of the ICH guidelines. Although the ICH guidelines were developed mainly for the validation of analytical procedures primarily based on the analyte being in solution, it was found possible to apply them successfully to the validation of a reflectance NIR intact tablet assay.

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