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Tablet identification using near-infrared spectroscopy (NIRS) for pharmaceutical quality control

L. Alvarenga [a], D. Ferreira [a], D. Altekruse [b], J.C. Menezes [a], D. Lochmann [b,*]

[a] Department of Biological and Chemical Engineering, Instituto Superior Técnico, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal
[b] Merck Serono Pharmaceutical Analytical Laboratories, Merck KGaA, Frankfurter Strasse 250, 64293 Darmstadt, Germany

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ABSTRACT

A need for more reliable and faster analytical methods for the identification of the active pharmaceutical ingredient (API) in finished pharmaceutical products is launched by the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, Q6A (1999). The use of infrared spectroscopy is suggested as a means to obtain specific identification. Near-infrared spectroscopy (NIRS) is a reliable method that offers important advantages for the large-scale production of tablets, such as high-throughput and accurate multiparametric data collection. Despite the grown number of reported NIRS identification methods, only a few methods have been approved by the regulatory authorities, which might be due to difficulties on clearly presenting the methods in official documents and audits. Motivated by the lack of clear protocols for the NIRS method's development, here we propose a process for building reliable identification NIRS methods.

For illustration purposes, a method is described for the identification of API in coated tablets containing 2%, 4% and 8% of thiamazole. The method described was successfully validated according to the International Conference on Harmonisation (ICH) of Technical Requirement for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology, Q2 (2005). The described method was subsequently approved by European national authorities and thus is suitable for use in cGMP environment.

1. Introduction

NIRS enables very fast and non-destructive multi-constituent analysis of virtually any matrix. Spectra can be collected directly *in situ* by using fibre optic probes, but other sample presentation modes are also available (e.g. cups, vials and custom-made sampling accessories). One single spectrum includes chemical and physical information on the sample. These are some characteristics that make this technique very interesting for pharmaceutical quality control, as reviewed by Reich [1].

A growing number of NIRS applications in process monitoring [2,3], reaction monitoring [4] and quality control [5] have been reported in the last decade. Nevertheless, only a few NIRS methods have gained regulatory approval yet. The main reason for this is believed to be related to the non-separative multivariate character of NIRS methods, when compared, e.g. with chromatography. Moreover, NIRS involves the use of sophisticated mathematical techniques for calibration, by opposition to the ability of mid-IR to identify samples by inspection of patterns on the spectra. In fact, identification with NIRS is based on comparing spectra with objective mathematical algorithms; hence it is expected to be more reliable. Resistance towards NIRS methods acceptance might be related with the difficulties encountered in clearly presenting them to the regulatory authorities.

At present, NIRS-based identification methods have their major role in the beginning of the production process, aiming at raw materials [6–8], and in the end of the production chain, testing the final product [9–12]. The identification/qualification of raw materials is a common practice in pharmaceutical industry. These are normally relatively different substances, in terms of their chemical nature. In this case, an NIRS identification method is based on a "fingerprint" approach, which may involve the use of the complete spectral range. For manufacturing of medicaments, all ingredients must be reliably identified and within this scope identification of pharmaceutical excipients has been reported [13]. The more challenging API-specific identification in final solid dosage form, formulated with several excipients, is more complex as it requires a more "rational" approach, based on understanding the specific spectral

* Corresponding author. Tel.: +49 6151 72 8569; fax: +49 6151 72 3155.
E-mail address: dirk.lochmann@merck.de (D. Lochmann).

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features and the use of chemometrics techniques to enhance that information.

The API identification in tablets has important applications in the pharmaceutical industry, both at the early product development stages and also later in routine productions. The developed methods can be challenged, for example, during clinical trials: when verum and placebo tablets are included, identification tests ensure that the clinical trial materials have been correctly packaged. Interesting studies measuring clinical trial tablets directly through the blister packaging have been presented [9,11]. Identification methods are also useful as a step before a quantification method can be performed [10,14]. Dempster et al. [11] achieved identification of 5%, 10% and 20% (w/w) API tablets, but concluded that the 2% (w/w) API tablets were indistinguishable from the placebo. De Maesschalck et al. [15], using a new supervised classification approach based on partial least squares beta classification (PLSBC), presented a model that was able to correctly identify clinical studies tablets with an API content of 1%, 2% and 3% (w/w) using NIR transmittance spectra. This new classification approach is also able to quantify the probability of misclassification, giving a measure of robustness. Using the same method together with the principle of data augmentation using both reflectance and transmittance modes, Van den Kerkhof et al. [16] suggested a way of constructing more robust classification models with few batches available. The NIRS detection limits depend on spectral selectivity. Pharmaceutical APIs and excipients have typically very different chemical natures and their signals appear in different regions in the NIR spectra. This makes NIRS a very sensitive technique for identification of APIs in low-dosed pharmaceutical formulations.

NIRS also is of great interest for the Process Analytical Technology (PAT) initiative driven by the United States Food and Drug Administration and the major pharmaceutical companies. One of the paradigms that is described in the guidance document is real time release (RTR). Skibsted et al. [17] presented practical examples within the RTR framework of using near-infrared and process data obtained from a tablet manufacturing process. In terms of quality check of final products, Herkert et al. [12] reported a NIRS-based method for the 100% on-line identity check of pharmaceutical products on the packaging line.

The direct identification of specific bands in NIR spectra is difficult or even impossible. These spectra are thus worked out by chemometrics tools. When the development of an identification method for tablets is based on the API detection and on a profound understanding of the spectroscopic and chemometrical data, the limits of detection can be pushed further and a more robust and specific method can be developed. NIRS implies walking a different path for both pharmaceutical companies and regulatory authorities. The knowledge generated during a well-planned method development strategy is also very valuable for the presentation of the NIRS method to the representatives of the regulatory authorities during an audit or when writing official documents.

The challenge inherent to the development of robust identification methods for low-dosed tablets is on the detection of the API specific spectral regions. NIR spectra are typically composed of broad overlapping peaks containing chemical and physical information on the sample. A spectrum taken of a formulation will contain added contributions from each component of the mixture. In low-dosed formulations, the excipients dominate the spectral information, representing a high risk for misidentifications of other products based on the same formulation. Additionally, it is often the case in pharmaceutical industries that the same formulation matrix is shared by different products. Thus, the detection of API specific spectral regions is essential for the development of valuable NIRS-based methods.

Although serving different purposes it can be said that the construction of an identification method in an industrial environment is less stressful and time-consuming comparing to quantification methods. The development of quantification methods depends on the availability of under- and over-dosed samples that the production operators are usually reluctant to produce since they are "out-of-specification" products. These extra samples must also be analysed with the reference method, which can be very time consuming. It can take several months to collect suitable calibration samples for a quantification method. For identification purposes, however, the observed variability within specification lots is high enough and a method can generally be developed when only commercial samples are available, which is faster and cheaper.

Recent guidelines launched by the European Medicines Agency (EMEA) [18] and the International Conference on Harmonization (ICH) Q6A [19] generated new needs in terms of the quality control of final products in pharmaceutical industry. These include the identification of the drug substance in the final drug product. A single chromatographic retention time, which proved to be sufficient for identification in the past, is no longer regarded as a sufficient proof of the presence of an active pharmaceutical ingredient (API), because it is not regarded as being specific. The use of infrared spectroscopy is suggested by ICH Q6A as a means to obtain specific identification. Near-infrared spectroscopy (NIRS) is a reliable method that offers important advantages for the large-scale production of high quality tablets. Meeting these guidelines, a strategy for the development and validation of safe and robust identification methods for tablets is presented and further illustrated with the example of a coated solid dosage form (thiamazole tablets).

2. Materials and methods

2.1. NIRS analysis

NIR spectra were recorded in transmission mode using a Vector 22 N-T FT-NIR Spectrometer (Bruker Optik GmbH Karlsruhe, Germany) suited for both transmission and diffuse reflection analysis and equipped with a 30-position automatic sample tray. Powder samples were measured poured into a glass vial that was directly placed on the sampler tray. Tablet samples were measured without any sample preparation using shape-specific sample holders placed on the sampler tray. Background reference was spectralon. The method was built with transmission spectra collected with optimised parameters: 50 scans at a resolution of 8 cm^{-1}. Spectra were visualised and chemometrically processed using OPUS Version 5.5 (Bruker Optik GmbH Karlsruhe, Germany). The chemometrics procedures are discussed ahead.

2.2. Samples

The developed identification method was aimed at solid dosage formulations containing 2%, 4% and 8% (w/w) of thiamazole (1-methyl-1H-imidazole-2-thiol; empirical formula: $C_4H_6N_2S$). The formulations are presented in the form of 250 mg convex round-shape film-coated tablets with a diameter of 9 mm and a score embossing in one of the faces. The formulation contains lactose as the major excipient. Coating follows a colour code for the dosage. The samples were obtained from the production site of Merck KGaA (Darmstadt, Germany).

API and excipients in powder were obtained regarding the quality requirements of the current European Pharmacopoeia.

Table 1
Summary of method parameters and method validation results

Method parameters	
Variable range	9300–8750 cm^{-1}
Spectral pre-processing	Vector normalization 1st derivative (21-points Savitzky–Golay smoothing)
Identification algorithm	Spectral distances in the design space
Method validation	
Specificity	Type I errors (false positives): 0 Type II errors (false negatives): 0
Robustness	All robustness set samples correctly identified

2.3. Calibration and validation sets

The data set was divided in calibration and validation sets after having all measurements collected. This division was based on chemometric analysis, such as principal component analysis (PCA) and group statistics information of the whole sample set, as well as on batch information, such as hardness, water and API content. The calibration set consisted on 250 measured samples from 25 different batches. The validation set consisted on 150 measured samples from 15 different batches.

2.4. Method parameters and validation

A summary of the method parameters and validation results are shown in Table 1. Thresholds were set in an iterative way with the library validation, so as to build robustness into the method. The final values are 0.15, 0.21 and 0.64 respectively for the 2%, 4% and 8% (w/w) API groups.

2.5. Specificity tests

Specificity tests were performed with matching placebos for each of the three dosages of thiamazole, i.e. excipient tablets with each of the three different coatings. Placebo tablets were an honours gift from Dr. Matthias Fischbach of the pharmaceutical development department of Merck KGaA (Darmstadt, Germany). Samples from 30 different solid dosage forms produced by Merck KGaA (Darmstadt, Germany) were also analysed to test the specificity of the method.

2.6. Robustness tests

Robustness tests were performed towards tablet horizontal rotation and tablet face presented to the light beam. Eight spectra were collected from the same tablet, rotating the sample 45° horizontally between consecutive measurements.

Robustness of the method towards water oscillations in the sample was also tested. Storing normal samples for 2 years in controlled conditions (25 °C with 60% relative moisture and 30 °C with 65% relative moisture) produced samples with about 1% higher water content.

3. Results and discussion

3.1. Strategy for API identification with NIRS

Meeting the new challenges launched in 2003 by the EMEA [18] and the ICH Q6A [19] for quality control in the pharmaceutical industry, a strategy for successful development and validation of NIRS-based identification methods aimed at oral solid formulations is presented, which can be seen in Fig. 1. The strategy comprises a systematic procedure of two iterative processes of data understanding leading to a robust method, which (1) is valid according to ICH Q2 [20], (2) can be used in a common technical document (CTD) and (3) is easily defendable in an audit, due to its underlying clear rationale.

This systematic procedure is illustrated in detail with the development and validation of an identification method for thiamazole tablets. These represent a serious challenge, since they are coated

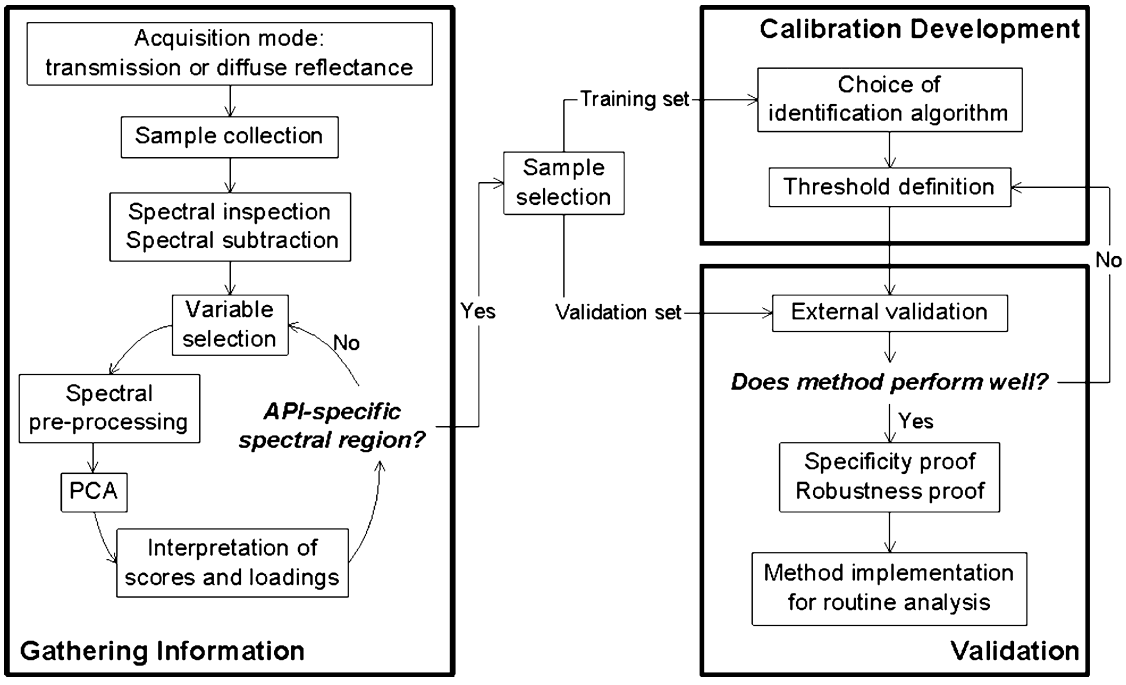

Fig. 1. Strategy for the development and validation of NIRS-based methods for API identification in low-dosed tablets.

tablets with a low content of API. The described method was approved by EMEA.

3.2. Selecting the appropriate measuring mode

Sample information in near-infrared region is usually collected as an absorption spectrum through transmission or diffuse reflectance mode. A decision between which one to adopt has to be made. The answer depends on the absorption properties and scattering characteristics of the sample and also on the target parameter, which has to be suitable for NIRS determination. In the case of intact tablets analysis, thickness and composition have to be taken in account.

In NIRS transmission, the light source and the detector are on opposite sides of the sample; the light reaches the sample from the side of the source, passes through it and is collected by the detector on the other side. The intensity of the light beam reaching the detector follows basically the Beer's law, which means that it depends on the path length. This technique has two very desirable characteristics. First, it represents a larger volume of the sample scanned, what provides a better representative spectrum of the entire tablet. Second, it can improve the accuracy, precision and sensitivity in the assays [21,22]. Since in transmission the incident beam passes through the sample, there is a decrease in the scattering effects from embossings and other markings common on pharmaceutical tablets. Also problems related with the inhomogeneity of the material and sample positioning have less effect on the resulting spectra. In fact, lower detection limits can be achieved by transmission rather than by reflectance. This can be important in case where the target compound of interest is present in low dosage levels. Coated tablets do not generally represent a problem to transmittance analyses since the spectrum is due to the whole tablet.

The major drawback of transmittance mode is the significantly narrower wavelength range available (12500–6000 cm^{-1}). In the less energetic region of the NIR spectra, too little light penetrates the tablet and therefore the detector signal becomes too noisy. Therefore, usually only the second and third overtone bands are successfully measured. This drawback is mostly overcame, since most APIs possess aromatic rings, which absorbance bands lay between 9090 and 7690 cm^{-1}, and most excipients are aliphatic. Useful API information can hence be obtained in this narrow wavelength range available. Limitations can be observed with very thick tablets because transmittance spectra may not contain enough information to construct reliable models.

In NIRS reflectance, the light source and the detector are at the same side of the sample; the light reaches the sample from the side of the source, scans the surface of the sample and is reflected back to the detector. The major advantage of the reflectance mode is the available wavelength range, which can be extended further into the infrared region (12500–400 cm^{-1}). Reflectance NIR spectra represent the thin peripheral layer of the sample. Beer's law is not followed, so no relation between the path length and the absorbance intensity can be made. Spectra can change considerably as physical properties of the sample vary (particle size and/or shape, surface roughness, packing density, sample thickness, hardness, etc.), due to the high influence of scattering. With reflectance mode it is previously assumed that the sample is homogenous. These pre-requirement most of the time cannot be assured and can lead to false results. Good examples of non-homogenous samples are coated tablets. If the target compound of interest is the tablet coating, reflectance is the indicated mode. On the other hand, if the object of the model is in the core of the tablet, it may be difficult to work with spectra dominated by information on the coating.

It is difficult to give a general rule for the choice of the measurement mode. Feasibly studies should always be performed before making a final decision. During these studies, sample presentation and minimum number of scans for efficient analysis should also be fixed [23].

3.3. Spectral inspection, spectral subtraction and variable selection

The direct assignment of bands to specific functional groups in the molecule is not simple in NIR, due to the strong overlapping of broad peaks. A NIR spectrum is the complex result of overtone absorbances and combination of absorbances. Nevertheless, the possible understanding of the spectral signals helps the rational selection of variables, i.e. wavelengths. The rational selection of the wavelength region on which the method will be based is crucial for the development of specific and robust identification methods. There are three main reasons for this; the first two are related with the nature of solid dosage forms: API possibly in low dosage added to a commonly used excipients mixture. The third reason for the careful selection of wavelength region is to avoid water active regions.

The key to build a robust identification method for solid dosage forms and avoid false positives is then to base it on the API information present on the spectra. Only spectral ranges related to the API should be considered for variable selection. Although the identification of these API-specific spectral ranges can be very challenging, the aromatic nature of most APIs can be an answer to this problem. Aromatic compounds show high absorbances in NIRS, which makes them suitable for this technique. Prior knowledge of composition and molecular structure of the API and the excipients mixture of the solid formulation is thus very valuable.

Water shows high absorption in the NIR region, which is due to the anharmonicity of O–H vibrations. Small variations of water content in the samples can have strong effects on the performance of the method. This parameter causes an extra variance within the data, which is not related to the main target. Since water effects cannot be removed or minimised by data pre-treatments, it is very important to construct models in water independent regions. Water exhibits multiple absorption maxima in the NIR region (10300, 8400, 6900, 5150 cm^{-1}). The position and width of these bands can be slightly shifted by temperature changes or hydrogen bonding between the analyte and the matrix. In tablets, the signal of water increases proportional to the compression force due to the densification of the powder and the greater interaction of NIR light with more molecules [24]. As compression pressure increases, water absorbance peak shift to longer wavelengths since there is an increase of hydrogen bonding within the compact [25].

With the objective of gathering information on the sample, pure thiamazole (API) and the excipients used to formulate the tablet were analysed by NIRS. These spectra are shown in Fig. 2. Looking at the thiamazole spectrum, the absorption band at 5500–6500 cm^{-1} can be originated in the overlapping of the first overtones of C=C and C—H, with the two peaks in 8000–9500 cm^{-1} being the respective second overtones. The thiamazole (API) peak centred around 9050 cm^{-1} is of special interest. Since the excipients show low absorption in this region, they are not expected to interfere with this API peak, which turns this wavelength range into a potential API specific region. For correct assignment of absorption bands to functional groups in NIRS, see ref. [26].

In Fig. 3A are presented spectra of the three different solid formulations and the matching placebos. The effect of the API specific band identified in Fig. 2 is clearly seen in the region around 9050 cm^{-1} of spectra from 2%, 4% and 8% API tablets. To further investigate the effect of the API on verum spectra, matching placebo

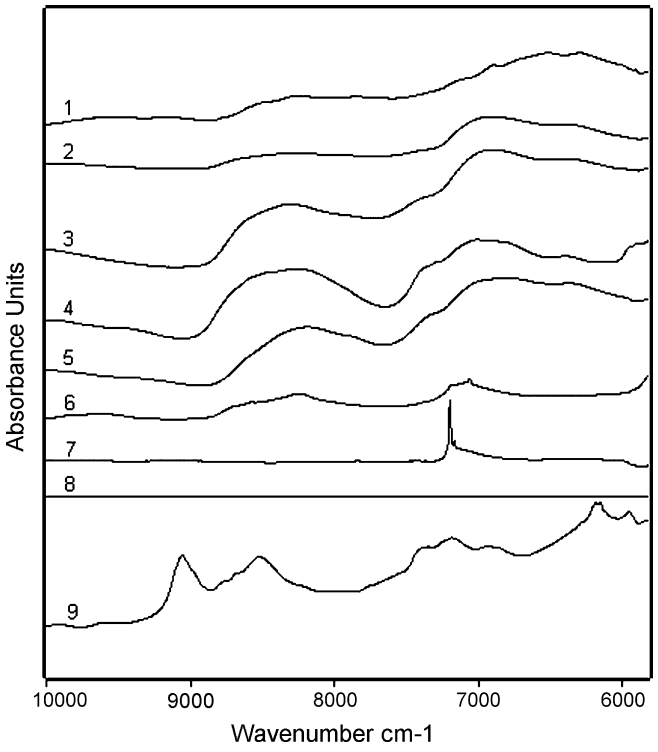

Fig. 2. Untreated NIR spectra of raw materials that formulate the thiamazole tablet, including pure thiamazole (API): (1) lactose; (2) corn starch; (3) sodium starch lycolate; (4) hydroxypropyl methylcellulose; (5) powdered cellulose; (6) magnesium stearate; (7) talcum; (8) silicium oxide; (9) thiamazole (API). Spectra were collected from powder samples in transmittance mode.

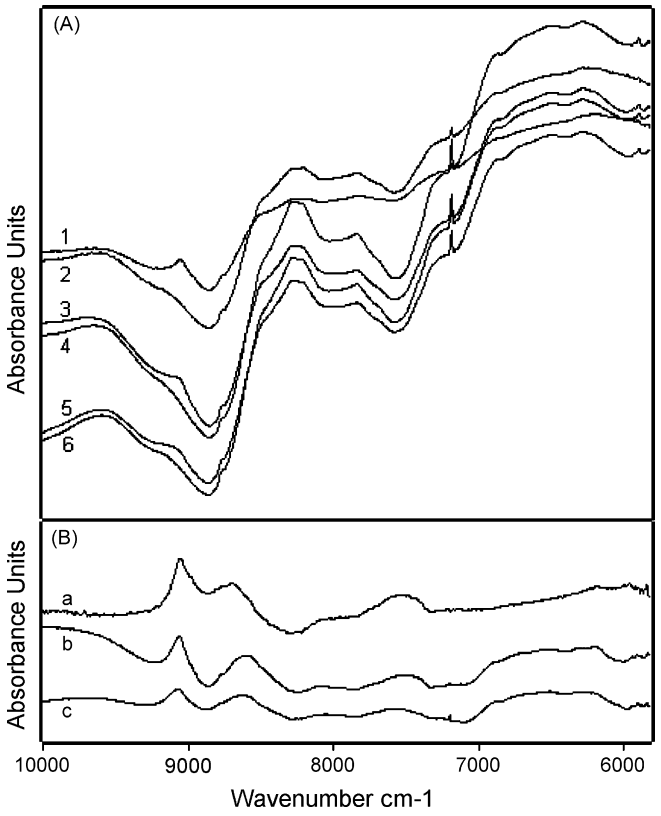

Fig. 3. Influence of the API on the tablet spectra. (A) Untreated transmission spectra of thiamazole tablets and matching placebos: (1) 8% verum; (2) 8% placebo; (3) 4% verum; (4) 4% placebo; (5) 2% verum; (6) 2% placebo. (B) Spectral subtraction: (a) (8% verum–8% matching placebo); (b) (4% verum–4% matching placebo); (c) (2% verum–2% matching placebo).

spectra were subtracted from each of the different dosages. The results of these subtractions are shown in Fig. 3B and are in accordance with the previous discussion. The subtraction spectra show a peak that corresponds to the API specific band at 9050 cm^{-1}. Both verum spectra and subtraction results show the presence of quantitative information for the API content in the referred range. Based on the given argumentation, it can be concluded that the range 9300–8750 cm^{-1} is an API specific region. Because it is also a water independent region, this spectral range was selected to build the identification method.

3.4. Spectral pre-processing

Spectral pre-processing are mathematical corrections that reduce, eliminate or standardize the effect of variable physical sample properties or instrumental effects on the spectra. Correct selection of spectral data pre-processing can significantly improve the specificity and the robustness of a model. Common pre-processing tools include multiplicative scatter correction (MSC) and standard normal variate (SNV), both used to correct scatter effects. Vector normalization and derivatives cancel the baseline offset. Derivatives also improve the resolution of overlapping bands, by enhancing the importance of peaks in relation to flat structures [27].

The selected wavelength range was treated with vector normalization and first derivative prior to chemometrical treatment. Since derivation also amplifies spectral noise, the Savitzky–Golay smoothing algorithm was combined with the derivation of spectra.

3.5. PCA, interpretation of scores and loadings

Along with the difficult or impossible univariate interpretation of NIR spectra, the multicollinearity found between wavelengths, build up the necessity to use multivariate data analysis techniques (chemometrics) to interpret spectral data. Pattern recognition methods are chemometric tools used in qualitative analysis. These can be “supervised” or “unsupervised”, depending on whether or not the class to which samples belong is given to the algorithm.

Principal component analysis is an unsupervised method of data compression and visualization widely used in NIRS technology. As an unsupervised classification method, no other information than the NIR spectra is given to the algorithm, so the clustering occurs without orientation. This mathematical tool resolves the multivariate spectral data into a much lower number of new uncorrelated variables that, through linear combinations, approximate the original spectra. The new variables are called principal components (PC) or factors. The first factor explains the maximum variability possible of the data and each successive new factor accounts for as much of the remaining variability as possible. This data compression helps with visualization, a scatter plot of the first two new variables being highly informative. Moreover, the first few PCs can be used as input to modelling tools that will not have to cope with highly multivariate data and, therefore, perform much better in terms of predictive ability and long-term accuracy (i.e. robustness).

In our example, a PCA of the region 9300–8750 cm^{-1} was accomplished to a data set consisting on spectra from the three verum with 2%, 4% and 8% (w/w) API and from the three matching placebos. Most of the spectral variability could be compressed to only two variables, with the third factor spectra representing mainly noise, as can be seen in Fig. 4. This figure compares factor spectra with spectra of pure API and tablets, in terms of the shape. The first factor captures the API information, as can be concluded from

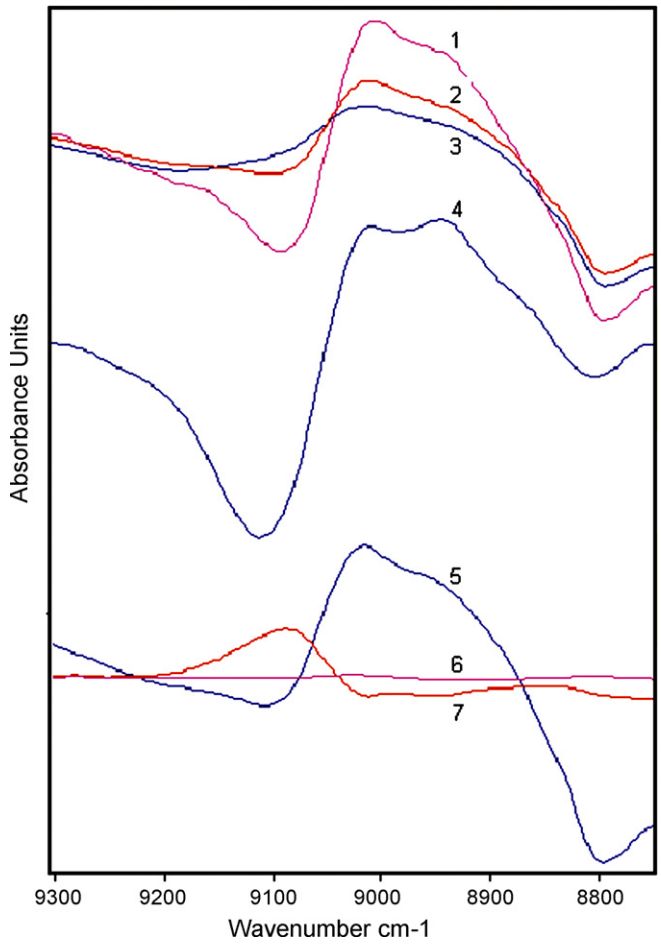

Fig. 4. Influence of the API on the factor spectra. Vector normalization and first derivative (with 21-point Savitzky–Golay smoothing) transmission spectra: (1) 8% tablet; (2) 4% (w/w) drug tablet; (3) 2% (w/w) drug tablet; (4) pure thiamazole (API) spectra. Factor spectra: (5) first factor (95% variability of the data set); (6) third factor (~0% variability of the data set); (7) second factor (5% variability of the data set).

the high similarity between this factor and the API spectra. This confirms that this spectral region is strongly related to the API.

A scores plot of the first two factors is shown in Figs. 5 and 6. The samples clearly cluster according to the API content: tablets with

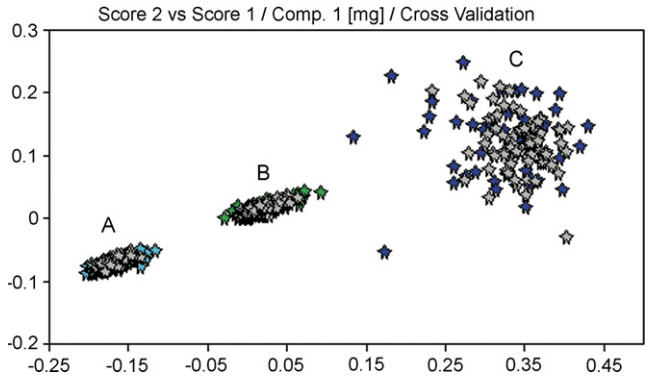

Fig. 5. Scores plot resulting from a PCA of the API specific region (9300–8750 cm^{-1}): (A) 2% tablets; (B) 4% tablets; (A) 8% tablets. Calibration set is represented in grey; validation set is represented in blue or green. Spectra were treated with vector normalization and first derivative (with 21-point Savitzky–Golay smoothing). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

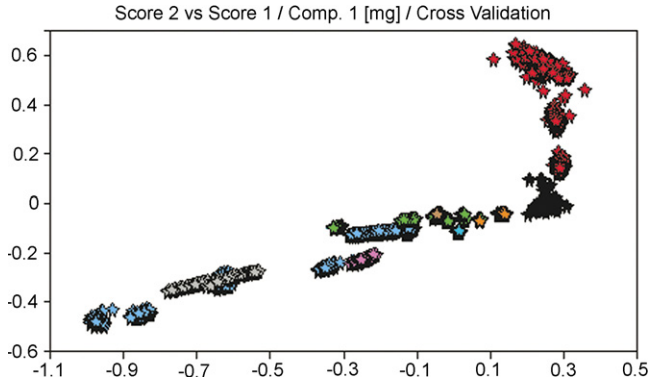

Fig. 6. Scores plot resulting from a PCA of the API specific region (9300–8750 cm^{-1}): the sample set includes thiamazole tablets [red], matching placebo tablets [black] and 30 other different products from Merck KGaA [other colours]. Spectra were treated with vector normalization and first derivative (21 Savitzky–Golay smoothing points). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

the same API content cluster together. The three different matching placebos cluster together (Fig. 6), which shows that the coating (which differs between different matching placebos) is not being modelled.

3.6. Sample selection

When enough information has been gathered about the system and before starting modelling, the sample set has to be divided in training set and validation set. The relative size of these sets may vary depending on the availability of samples, but the training set is in general not smaller than 2/3 of the whole sample set. To obtain suitable multivariate models, the selection of the training set is a crucial step. According to Næs et al. [28], the quality of the samples is more important than the number of samples. A moderate range of samples is usually easier to fit by a model than a large range and, with a good selection of samples, most of the variability of a bigger set can be conserved. A good sample set includes variation as large as possible in all directions and limited to the region of interest.

The division between training set and validation set was done at the batch level. Batches with a higher variability were chosen to compose the training set, being the other batches left for the external validation. The variability was evaluated based not only on the hardness, API and water content (batch certificates) but also on the PCA analysis, to avoid the dimensions collinearity of the data set [28]. In Fig. 5, a PCA to the API specific WL range (9300–8750 cm^{-1}) of the whole data set, which consisted on 250 spectra collected for 25 different batches of the three different dosages of thiamazole, is shown. Samples are selected that show biggest variation in the most dominating directions (PC) by visual inspection of the scores plot, which means in Fig. 5, samples that appear in the periphery of the cluster.

3.7. Choice of identification algorithm

Discriminant analysis or supervised classifications are the names given to chemometrical methods for identity/quality analysis. Most of these classification methods can operate either in wavelength space or in a dimension-reduced factor space and their ultimate goal is to establish mathematical criteria for parametrizing spectral similarity. Comprehensive libraries of spectra that represent the natural variation of each product have to be constructed in a "calibration" process, with similarity between spectra being expressed by a correlation coefficient or a spectral distance. In the

software OPUS used in this study, the classification methods are based on the measurement of spectral distances, which can be measured in wavelength space or in the dimension-reduced space [29]. The second option was chosen and an identification method was built based on the two first factor spectra (Fig. 4).

3.8. Thresholds definition

Another crucial step in discriminant analysis is the setting up of limits to the different groups of a library, called "thresholds". The threshold can be physically understood as an external boundary to a number of samples relatively close to each other and representative of a group. The choice of the appropriate value for the threshold ensures adequate selectivity of the library. Too low a threshold can result in spectra belonging to the same class being incorrectly classified. Too high a threshold can lead to confusion between substances with similar spectra. Choosing the appropriate threshold must be done simultaneously with the external validation of the library in an interactive manner: the threshold is successively changed until a good performance towards the validation set of samples is achieved [6].

3.9. External validation

The external validation of the method is performed with the samples of the validation set, i.e. samples that are from independent batches from those used to calibrate the method. It is realised simultaneously with the threshold setting, to assure a correct selection of this parameter. The method was challenged with 150 certified samples from each of the three different thiamazole formulations. All samples were correctly identified.

According to ICH Q2 [20], proof of specificity and robustness are necessary to validate an identification method.

3.10. Specificity proof

To test the specificity of the method for thiamazole products, potential challenges are presented to the library. The specificity proof set was composed by two groups of samples. The first group of samples included matching placebos for the three different dosages. The second group included samples from 30 different tablet formulations coming from the pharmaceutical production plants of Merck KGaA (Darmstadt, Germany).

The library rejected all the samples from the specificity proof set and its specificity was then proved. In Fig. 6 is presented a scores plot from a PCA where the specificity proof set was included. In this plot, the distance of the different products from the thiamazole samples shows that this identification method clearly differentiates them, which guarantees no identification errors.

3.11. Robustness proof

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations of the operational parameters. It provides an indication of its reliability during normal usage [20]. Since NIRS does not often involve sample pre-treatment, the only operational parameters that can affect the results are those inherent to the spectrometer, the environment (unless it is controlled), sample quality (e.g. water content) and the presentation of the sample to the light beam. Some typical challenges for the robustness of a method are listed by Broad et al. [23]. Borer et al. [30] evaluated the influence of various parameters on the performance of NIRS based libraries. Tablet orientation, especially for embossed tablets, and inter-day variability, were found to be two important factors on the ability of NIRS to discriminate between dose-levels of tablets. Inter-day variability was already included in the library which was built over several days.

To evaluate the effect of sample orientation on the performance of the method, two parameters were investigated in the thiamazole example: tablet horizontal rotation in the sample holder, and tablet face. Eight spectra were taken from the same tablet, rotating the sample horizontally 45° between consecutive measurements. Each time, a different surface was exposed to the NIR light beam. The influence of sample rotation on the spectra was visible on the baseline shift. However this effect is removed or minimised by the spectra pre-treatment used to build the model. Thus, the model correctly identified all the spectra, which proves its robustness toward sample positioning. Samples used to build the model were measured with random position and so variability due to sample position was included in the training set.

The thiamazole tablets are designed with an embossed score on one side of the tablet that is intended to permit the patient or healthcare provider to split the tablet in order to reduce the dosage. In relation to tablet face, two geometries are then possible: score up and score down. The training set was constituted of spectra taken with score down because it had a larger signal-to-noise. Now, a total of 100 spectra with score up from the three different API dosages were collected and all were correctly identified.

Due to the possible effects of water on the performance of NIRS-based methods, water modified samples were also included on the robustness tests. After production, the thiamazole tablets contain approximately 4% of water. However, the water content can be modified by stressing the samples in acclimatised rooms. Thiamazole 2% and 4% (w/w) samples were stored for two years in two different acclimatised rooms: 25 °C/60% relative humidity or 30 °C/65% relative humidity. After this time, the samples gain 1% in water content. Stressed samples spectra were not altered in the range used to construct the method (9300–8750 cm^{-1}), spectra from stressed and unstressed samples were indistinguishable (data not shown). The same spectra showed effect of water content in a water specific spectral region, which proves the real uptake of water. This investigation showed that the method was built using a water independent spectral range. All water modified samples from this investigation were correctly identified by the method.

In case of failure of a method while robustness tests one of two actions should follow. If possible and reasonable, failed samples should be included in the training set, building robustness into the method [18]. Otherwise, the tested parameter should be documented and controlled during the normal usage [20]. Robustness is somehow related to the sample selection: the fewer the number of variables likely to affect the performance of the method, the smaller the number of samples required for the training set.

4. Conclusions

A more systematic and rational strategy for development and validation of NIRS identity methods was presented that encompasses the current guidelines. To prove and illustrate the proposed strategy, coated tablets containing thiamazole as the API were studied. Focusing on the specificity and robustness of the method it was proven that not the fingerprint of excipients or other spectral artefacts but the API information dominates the prediction of the thiamazole content and thus the identification of the API in the finished product. The method presented was successfully validated regarding ICH guidelines and was approved by European regulatory authorities. The presented strategy provides thus a safer way of developing NIRS identification methods, which can be used in GMP environments like the pharmaceutical industry.

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