RIVM Report 670400003/2002

Verification of the identity of pharmaceutical substances with near-infrared spectroscopy

P.W.J. Caspers, M.J. Vredenbregt, R. Hoogerbrugge,
D.A. van Riet-Nales and D.M. Barends

This investigation has been performed by order and for the account of the National Institute of Public Health and the Environment, within the framework of the project *Advanced Analytical Methods* (Project 670400).

RIVM, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
Telephone: +31 30 274 91 11
Telefax: +31 30 274 29 71

Abstract

It has been investigated whether NIRS can be applied as a stand-alone method for verifying the identity of pharmaceutical substances in a pharmaceutical setting, what the minimum requirements are, and how these methods should be validated. Concluded is that NIRS is acceptable as stand-alone method. However, the requirements are a careful construction of the spectral library and development, validation and maintenance of the method. To validate the specificity and reliability, such NIRS methods should be challenged with a rationally composed set of other substances. The use of the chemometric algorithms ‘wavelength correlation’ and ‘maximum wavelength distance’, that are based on standard mathematical formulae, applied on the whole near-infrared range (1000 – 2500 nm) is preferred. Additional guidance is provided in this report. This can be used by both the pharmaceutical industry and competent authorities. It has already been used to formulate an European draft guideline that covers the application of NIRS in the pharmaceutical industry.

Contents

Summary

Near-infrared spectroscopy (NIRS) provides rapid and environmentally friendly analyses that can be conducted in production areas without the need for highly trained personnel. It is suitable and especially advantageous to replace repetitive analyses that are frequently done at a given location, such as the single-container verification of the identity of incoming pharmaceutical substances, particularly if supplied in many containers. An increasing number of pharmaceutical companies want to use NIRS for this purpose. For the development, use, and maintenance of NIRS methods for such applications, guidance additional to that of the European Pharmacopoeia (Ph Eur) is required.

We investigated whether NIRS can be applied as a stand-alone method for verifying the identity of pharmaceutical substances in a pharmaceutical setting, what the minimum requirements are, and how these methods should be validated.

Tentative minimum requirements were defined on the basis of experience and literature review, and then they were evaluated. These requirements concern the wavelengths to be used, acceptable spectrum pretreatments, the number and nature of the samples to be used for building the spectral library, acceptable chemometric algorithms for comparing the spectra, validating the method, and maintaining it.

A twofold release procedure for pharmaceutical substances was created. One is based on the chemical identification methods in the monographs of the Ph Eur, along with any relevant, additional conventional methods for relevant properties of a substance other than the chemical identity. The other release procedure, involving a NIRS identification method, was developed at the RIVM.

The comparison of these methods shows that NIRS is acceptable as a stand-alone method for verifying the identity of pharmaceutical substances. The requirements are a careful construction of the spectral library and the development and validation of the method. To validate the specificity and reliability, such NIRS methods should be challenged with a rationally composed set of other substances. The use of the chemometric algorithms ‘wavelength correlation’ and ‘maximum wavelength distance’, that are based on standard mathematical formulae, applied on the whole near-infrared range (1000 nm – 2500 nm) is preferred. As a result of this investigation and the evaluation of the tentative minimum requirements, we formulated a technical report with the minimum requirements for applying NIRS to verify the identity of pharmaceutical substances.

Samenvatting

Nabij-infrarood spectroscopie (NIRS) biedt de mogelijkheid tot snelle en milieuvriendelijke analyses, welke uitgevoerd kunnen worden op de werkvloer door niet-analytisch opgeleid personeel. Het is geschikt en vooral voordelig ter vervanging van standaardbepalingen die frequent uitgevoerd moeten worden op een bepaalde locatie, zoals bijvoorbeeld de per verpakking uit te voeren verificatie van de identiteit van binnenkomende farmaceutische grondstoffen, met name indien geleverd in veel eenheidsverpakkingen. Een toenemend aantal farmaceutische bedrijven willen NIRS hiervoor gebruiken. Voor de ontwikkeling, het gebruik en het onderhouden van zulke NIRS toepassingen zijn richtlijnen aanvullend op de Europese Farmacopee noodzakelijk.

Onderzocht is of NIRS gebruikt kan worden als enige methode ter verificatie van de identiteit van farmaceutische grondstoffen in de farmaceutische industrie, aan welke randvoorwaarden zo'n toepassing zou moeten voldoen en hoe deze gevalideerd dient te worden.

Concept randvoorwaarden zijn opgesteld, op basis van ervaringen en literatuuronderzoek, en geëvalueerd. Deze randvoorwaarden betreffen het gebruikte deel van het nabij-infrarood spectrum, de toegepaste spectrum voorbehandeling, het aantal en aard van de monsters gebruikt voor het samenstellen van de referentiebibliotheek, de toegepaste chemometrische algoritmen ter vergelijking van de spectra, en de validatie en onderhoud van de toepassing.

Een paralelle vrijgifte van farmaceutische grondstoffen is opgezet: één vrijgifte met de identiteitsbepalingen zoals vastgelegd in de Monografieën van de Europese Farmacopee, met, indien relevant, aanvullende conventionele methoden ter bepaling van kwaliteitaspecten anders dan de chemische identiteit, en een vrijgifte met een door het RIVM ontwikkelde NIRS toepassing.

Vergelijking van beide vrijgifte methoden gaf aan dat NIRS aanvaardbaar is als enige methode ter bepaling van de identiteit van farmaceutische grondstoffen. Een zorgvuldige samenstelling van de referentiebibliotheek en ontwikkeling en validatie van de NIRS toepassing is hiervoor vereist. De toepassing dient ten aanzien van specificiteit en betrouwbaarheid gevalideerd te worden met een rationeel samengestelde validatieset van andere grondstoffen. Het gebruik van de op standaard mathematische formules gebaseerde

algoritmen ‘wavelength selection’ en ‘maximum wavelength distance’, toegepast over het gehele nabij-infrarood spectrum (1000 nm – 2500 nm), heeft hierbij de voorkeur.

Op basis van de resultaten van dit onderzoek en evaluatie van de concept randvoorwaarden is een Technisch Rapport opgesteld met randvoorwaarden voor de toepassing van NIRS bij de verificatie van identiteit van farmaceutische grondstoffen.

Abbreviations

BCAP	A cluster analysis application from Buchi
CI	Conformity index
EDQM	European Directorate for the Quality of Medicines
EMEA	The European Agency for the Evaluation of Medicinal Products
LGO	Laboratory for Quality Control of Medicines, RIVM
LOC	Laboratory for Organic Analytical Chemistry, RIVM
MD	Mahalanobis distance
MIR	Mid-infrared
MSC	Multiplicative scatter correction
MWD	Maximum wavelength distance
NIR	Near infrared
NIRS	Near-infrared spectroscopy
PCA	Principal components analysis
SIMCA	Soft independent modelling of class analogy
SMV	Spectral match value
SNV	Standard normal variate transformation
WC	Wavelength correlation

Glossary

Ambiguous conclusion	The sample is considered identical to more than one substance present in the reference library.
Calibration	The process of creating a model relating two types of measured data; for NIRS, a model that relates concentrations or properties, i.e. identity, to absorbance spectra for a set of reference samples (the reference library or the calibration set).
Calibration set	The set of samples used for creating the calibration model.
Change control protocol	A protocol listing potential future changes in the method and the actions considered necessary to prove that the reliability of the method has not been diminished after these changes.
Change control test	A test used to demonstrate unchanged reliability after a method has been changed.
Chemometrics	Mathematical pattern recognition methods to compare data, i.e. spectra.
External validation I	At least one spectrum of an independent batch (this is a batch that has not been included in the reference library) of each substance or form included in the application is tested with the application. Each should be identified or qualified unequivocally.
External validation II	This validation is performed with all the spectra from other substances or properties that are present in the database and that are not included in the application. All these other spectra should give a ‘no match’ result.
Internal validation	The batches that are included in the reference library are validated on selectivity to each other. All included batches should be identified/qualified unequivocally, without conflicting results.
Model updating	Incorporating new substances or new sources of variance, which occur in practice, into the classification model to expand the application, to make it more robust, and to maintain its applicability.
NIRS application	The whole setting of one or more NIRS methods to analyse substances.

NIRS method	The model fixed by definition of the measurement technique and the spectra pretreatment, including wavelengths and the chemometric algorithm with threshold.
No-match conclusion	The sample is not considered identical to any substance in the reference library.
Pass conclusion	The sample is considered identical to a substance or form in the reference library.
Performance verifications	Tests to control the instrument performance.
Pretreatment	Processing of the spectral data, with mathematical or other techniques, before the spectra are compared with chemometrics.
Reference library	A database containing spectra of several batches of several substances or properties to be tested. Spectra of unknown samples are compared with this database.
Reference method	The conventional analytical method that is used to determine the concentration or property value of the samples.
Threshold	A limiting value for qualitative methods, which is decisive for a pass or a no-match conclusion.
Training set	The set of samples included in the reference library for one and the same substance or form.
Transflectance	A transmittance measurement technique where the light traverses the sample twice, the second time after being reflected from a surface behind the sample.
Validation set	Set of samples used in the validation of the application.

1 Introduction

1.1 Verification of identity of pharmaceutical substances

The rules governing medicinal products in the European Union state that, to meet good manufacturing practice (GMP), no substances used for the manufacture of medicinal products are released for use until their quality has been judged satisfactory by the Quality Control Department[1]. They state that the identity of a complete batch of starting materials can normally only be guaranteed if individual samples are taken from all the containers and the identity of each sample is tested. They state that it is permissible to sample only a proportion of the containers when a validated procedure has been established to ensure that no single container of starting material has been incorrectly labelled. Appropriate and by competent authorities approved specifications are laid down for each substance and should be met. Substances for which the qualities are described in the Ph Eur should always meet these pharmacopoeial specifications.

At present, it is common practice to test samples from the incoming materials for identity and other quality specifications. If the starting materials are supplied by certified suppliers, only the identity will be verified because these materials are accompanied by certificates of analysis that guarantee compliance to the specifications. Next, the identity of each container of a batch of incoming material is verified. To the present time, these tests have been performed with conventional, ‘wet chemical’ analytical methods in the Quality Control Laboratory. These procedures are very time-consuming and expensive; they often require the use and disposal of environmentally unfriendly chemicals and can delay production. This situation may contribute to noncompliance to the Rules and infer the verification of the identity of every single container.

The introduction of near-infrared spectroscopy (NIRS) analytical methods has provided a wholly different approach to verifying the identity of pharmaceutical substances, and it has many potential advantages.

1.2 Near infrared spectroscopy (NIRS)

The NIR ranges from 800 nm to 2500 nm (corresponding to a frequency range of 4000 cm^{-1} to 12 500 cm^{-1}), between mid-infrared (MIR) and visible light, and covers overtone and combination vibrations of the MIR range of -OH, -CH, -NH, and -SH groups. The intensity of the generally broadly overlapping NIR bands is weaker than the intensity of the fundamental IR bands by a factor of 10 to 100. Since the ratio of reflected light to absorbed light is high in the NIR range, the technique is particularly suited for diffuse reflection measurements. Transflectance and transmission measurements are also applied.

1.2.1 Chemometrics and pretreatments

Since NIR spectra contain both chemical and physical information, it is impossible to interpret them simply and directly. Differences in the spectra of substances cannot easily be related to differences in the properties (both chemical and physical) of these substances. Spectra should therefore be compared by mathematical pattern recognition methods. These methods, called chemometrics, are tools to reveal differences and similarities between spectra. The type of chemometric algorithm to be used on a spectrum depends on which property one wants to differentiate, and, consequently, on what information should be revealed.

The methods can be optimised by pretreatments. A pretreatment is a mathematical or other technique applied to the raw spectra before they are compared by chemometrics. It is possible, for example, to minimise physical effects on the raw NIR spectra with the appropriate application of suitable pretreatments. If the physical properties, e.g. particle size, are the subject or part of the qualification, then the physical effects, of course, should not be minimised[2].

1.2.2 Advantages and disadvantages of NIRS

Compared to conventional analytical methods, the advantages of NIRS applications are:

- The simultaneous determination of various properties of a substance in one spectrum;
- Simple sample preparation or collection, or even none at all;
- Measurement through transparent packaging materials like glass and some plastics;
- Measurement directly in the production area without the need of highly trained personnel;
- Nondestructive;

- Environmentally friendly (no disposal of samples, solvents, or reagents);
- Speed;
- The equipment is relatively small and not very expensive.

The most important disadvantages are:

- The development of an application is time-consuming and the maintenance often inconvenient.
- The method is not very flexible; its transferability to other equipment cannot be taken for granted yet.
- NIRS is a secondary method; the accuracy cannot be better than the accuracy of the reference method.
- Black box experience; the relation between the spectral information used and the property tested is often not clear.
- The reliability is not yet well defined for pharmaceutical applications.
- Because the technique is not very sensitive, it is less suitable for testing impurities and low-dose substances.

1.2.3 Applications

NIRS analysis has gained wide application in several fields of analysis during the last three decades. Methods have been developed especially for use in agriculture and the food industry[3]. Since the FDA accepted a NIRS method for testing the identity, assay, and water content of the active substance ampicillin trihydrate as an in-process release test in 1992[4], NIRS has been introduced more and more often as an alternative method in the pharmaceutical industry. Many applications have been developed and researched[3]. Examples are methods for moisture content, polymorphic form, particle size, and verifying the identity of raw materials. Other examples are methods for coating thickness, hardness, and assay of pharmaceutical products and in-process controls such as the moisture content of granulates, blend uniformity, coating thickness, hardness of tablet cores, and particle size of intermediates in manufacturing. There are advantages such as rapid measurement in the production and warehouse area of several properties in one spectrum that can be done by personnel who are not specifically analytically trained. These advantages make it a very interesting method. Several possible applications of NIRS in the analysis of pharmaceutical products are described and discussed in RIVM Report 670 400 002[5].

1.3 Verification of identity with NIRS

Using NIRS to verify the identity of substances for pharmaceutical use provides many advantages. Consequently, there is a strong desire and tendency to replace the conventional methods with NIRS. If a fibre optic probe is connected to a NIR spectrometer, we can directly verify the identity of materials in their original containers, without the need for sample collection, transportation, storage, identity analysis in the laboratory, and disposal of the collected samples and chemicals used for testing with conventional methods. In particular, when many verifications are required, for example, for incoming containers in a warehouse, NIRS is an advantageous option. In addition to information about the chemical identity, information about other important properties like particle size, polymorphic form, and moisture content can be derived from one recorded spectrum. NIRS analysis differs from most of the methods of the Ph Eur, which check only the identity and other quality parameters such as expected impurities. NIRS analysis may also indicate the presence, at macro levels, of unexpected contamination. NIRS also differs from the current widely accepted identification method of mid-infrared (MIR) spectroscopy. While MIR analysis is based on the visual comparison of the spectra, identification with NIRS is based on comparing the spectra by means of objective algorithms; hence it is expected to be more reliable.

A monograph on NIRS was included in the Ph Eur in 1997[6]. Regulatory authorities are now encountering the first applications for the use of NIRS analytical methods. These applications encompass the verification of identity as well as quantitative methods (assay, moisture content). An impressive increase of NIRS applications in the pharmaceutical industry is expected, and consequently the focus should be on the assessment of these methods. Reference to this monograph alone is not acceptable for the quality assurance of NIRS methods. The monograph contains merely technical and methodological guidance on the equipment, the preparation of the sample, the control of the instrument performance, and guidance on how to build a spectral library. With regard to validation, it only states that ‘the selectivity of the database to positively identify a given material and discriminate adequately against other materials in the database is to be established during the validation procedure’. There is no indication of how this validation should be addressed and what requirements should be met. Nothing is said about the maintenance of the method. In view of this, additional guidance is required.

1.4 Objectives

The objectives of this project were to investigate:

- Whether NIRS can be applied as stand-alone method for verifying the identity of pharmaceutical substances in a pharmaceutical setting;
- What minimum requirements should be met;
- How the validation of these methods should be addressed.

Specific questions that should be answered in this respect are:

- What are the requirements for the batches that are used for building the reference library?
- How many batches should be included in the library for each substance to be tested?
- Which chemometric algorithms are allowed, and which algorithm is preferable?
- Is pretreatment allowed and necessary, and which techniques are allowed?
- How should the thresholds for match/no match be defined, and are there minimum requirements?
- Can the reliability of verifying identities with NIRS be quantified?
- Should a certain method of validation be imposed, and if so, which one?
- Which challenges should be included in the validation?
- Is it acceptable that for validation not all expected challenges to the method are actually experimentally evaluated by recording and comparing its spectra?
- Should the method be validated for robustness, and if so, how?
- How should instrumental changes and changes in the method, including those of the reference library, be addressed?

Definition. Sometimes there is confusion about the term ‘identification’. In the monographs of pharmacopoeias with the given identification method, it means determination of the chemical identity. In the pharmaceutical industry, identification can also include differentiation of different physical properties of one chemical substance (e.g. particle size, polymorphic form, and viscosity). In the context of this report, ‘identification’ is the same as in the pharmaceutical industry.

2 General approach

A draft technical report, *Identification of active substances and excipients with NIRS*, contains tentative minimum requirements for all the aspects of the development, validation, and maintenance of a NIRS method used for verification the identities of pharmaceutical substances in a pharmaceutical setting. A twofold release procedure has been created to evaluate this draft technical report (Figure 2.1).

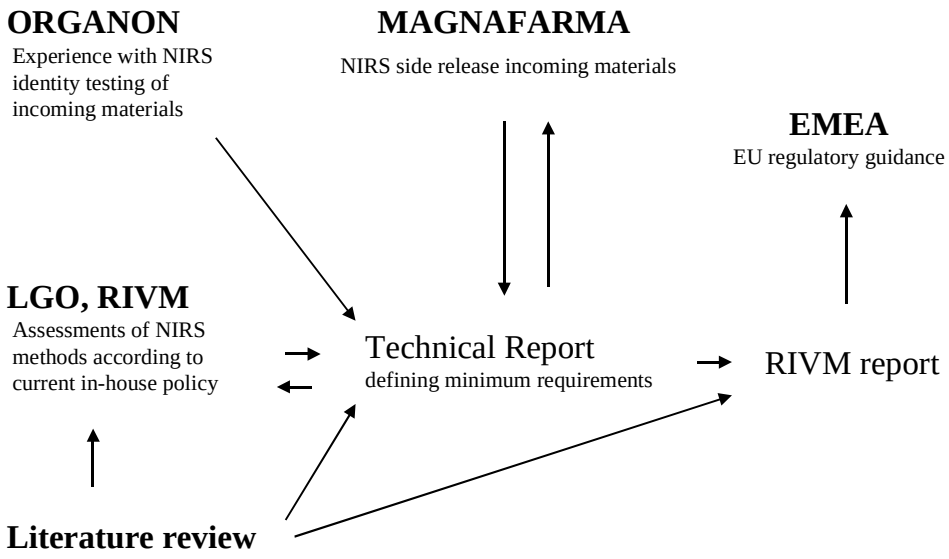


Figure 2.1. General approach of the study

First, we took inventory of the current scientific knowledge on identity testing with NIRS in a literature review. Second, experts' opinions on the application of NIRS in the pharmaceutical industry were obtained from the quality control department of Organon, Oss, the Netherlands, an innovative pharmaceutical industry with years of experience with NIRS. Based on the knowledge thus acquired and the experience and knowledge already present at the Laboratory for Quality Control of Medicines (LGO) of the RIVM, especially in the field of the regulatory aspects of NIRS methods, a draft technical report was formulated. The LGO accounts for the assessments of Part II of the dossiers for applications for marketing authorisation in the Netherlands.

These tentative requirements defined in the draft technical report were evaluated during the development of a NIRS application at the Laboratory for Organic Analytical Chemistry (LOC) of the RIVM and the subsequent comparison of this application with the conventional methods. The LOC covers the organic chemical analysis at the RIVM.

A twofold release of pharmaceutical substances was created: one release procedure based on the chemical identification methods of the monographs of the Ph Eur, with, if relevant, additional conventional methods for other, physical properties of a substance, and one release procedure with a NIRS identification application developed at the LOC.

The application was developed and validated in accordance with the draft technical report and common GMP practice, as it would have been done in a pharmaceutical setting. After development and validation, the application was challenged with samples that had already been tested at the quality control department of Magnafarma, a Dutch manufacturer of generics, who also supplied the samples. Herewith the conventional methods used at this control laboratory and the NIRS application at the LOC were compared, and subsequently the tentative minimum requirements defined in the draft technical report were evaluated.
It is emphasised that it was certainly not the intention to develop an optimum application; an evaluation of the tentative minimum requirements is preferably based on the experience with an application that, although in compliance with the report, is as far from optimal as possible while still allowed.

The results of the comparison of the release procedures and the subsequent evaluation of the draft technical report gave an indication of the suitability, reliability, and acceptability of the use of NIRS as a stand-alone method for verifying the identities of substances in a pharmaceutical setting, the minimum requirements for such an application and its validation, and also answers to the defined questions. With this information, the technical report was adjusted and subsequently used as a basis for the Dutch NIRS assessment policy and the coming European regulatory guidelines.

3 Literature review

This chapter presents a review of the international literature on several specific issues concerning the development, use, and maintenance of a NIRS application for verifying the identities of pharmaceutical substances.

Several methods for verifying the identity have been examined and are described in the literature (Table 3.1). Most of the relevant articles describe the development of a method, and they begin by listing the substances to be discriminated from each other. In these cases, a method that yields the best, and most reliable, discrimination of the substances is searched for. Appropriate performance of the method is then confirmed in a process of validation for specificity with the substances. An optimum combination of spectrum pretreatments and chemometrics, with suitable thresholds, is sought.

3.1 Pretreatments and chemometrics

A suitable combination of the applied pretreatment and chemometric technique should yield access to the information in the spectra that is necessary to discriminate among the various pharmaceutical substances to be verified (‘identities’) and other chemical pharmaceutical substances (‘nonidentities’) that should be rejected. The pretreatments, chemometric algorithms, and their combinations described in the reviewed literature are presented in Table 3.1.

3.1.1 Pretreatments

By applying appropriate preprocessing techniques, it is possible to minimise the physical effects on the NIR spectra[7]. If the physical properties, e.g. particle size, are the subject or part of the discrimination, then of course these contributions should not be minimised[2]. Several pretreatments are commonly used. These are mathematical treatments, such as using derivatives (first, second, or more) on the spectra, averaging over spectral ranges (smoothing), and the use of certain selected wavelengths or wavelength ranges only. Other pretreatments are normalisation and other techniques to correct for light scattering effects or baseline shifts. Examples of these pretreatments are baseline correction, standard normal variate transformation (SNV), and multiple scatter correction (MSC).

Table 3.1. Studies on NIRS methods for identifying pharmaceutical substances

Study	Substances/Methods	Results	Comment
Gerhäusser et al.[9]	Benzodiazepines PT: no/second-derivative/ WS CM: WC/MWD/PCA	Favourite method PT: second-derivative CM: PCA and WC	Several methods were compared
Candolfi et al.[12]	10 excipients Several PTs are combined with several CMs PT: BC/MSC/second-derivative/DT CM: PCA/MWD/TPF	Favourite method PT: SNV CM: MWD	Several methods were compared
Candolfi et al.[2]	Cellulose microcrystalline PT: SNV CM: PCA versus MWD	Model updating with MWD is straightforward; with PCA methods, more complicated	Original calibration set (n = 17) is extended and revalidated
Ulmschneider et al.[14]	5 active substances PT: second-derivative CM: WC	Unequivocal identification	
Ulmschneider et al.[11]	7 intermediates and actives PT: 1st derivative + WS + N CM: PCA plus BCAP	Unequivocal identification	Two spectrometers
Ulmschneider et al.[15]	9 active substances PT: 1st derivative CM: PCA	Unequivocal identification. Calibration sets are transferable	Calibration on three different meters.
Ulmschneider et al.[13]	2 starches, 5 sugars, 4 celluloses PT: NBC/1st derivative/ MSC/second derivative CM: PCA plus NIRCAL	Unequivocal identification. Method transferable to other spectrometer	
Yoon et al.[8]	15 common solvents PT: second-derivative CM: WC	Unequivocal identification	
Kramer et al.[10]	8 celluloses and cellulose ethers PT: MSC/1st derivative/WS CM: PCA versus SIMCA	Additional tests are required for some identifications	
Gemperline et al.[7]	10 substances, including 6 celluloses PT: no CM: MWD/SIMCA/MD	MWD suitable for identification, but not for purity	Comparison large and small calibration sets
Plugge et al.[4]	Ampicillin trihydrate Several parameters including identity, assay, and water content Celluloses CM: SMV, CI	Several parameters tested in one spectrum	Accepted as in-house release test by the FDA

BC: Baseline correction (detrending, offset), BCAP: cluster analysis module from Buchi, CI: conformity index, CM: chemometric method, DT: detrending, MD: Mahalanobis distance, MSC: multiple scatter correction, MWD: maximum wave distance, NBC: normalisation by closure, NIRCAL: cluster calibration module from Buhler, PCA: principal components analysis, PT: pretreatment, SIMCA: PCA plus cluster analysis (FOSS), SMV: spectral match value, SNV: standard normal variate transformation, TPF: triangular potential function, WC: wavelength correlation, WS: wavelength selection

Yoon et al.[8] found that, for discriminating several solvents, better results were obtained with the second-derivative spectra and with the use of the chemometric algorithm for wavelength correlation (WC) applied on NIR transflectance spectra. Third and fourth derivatives gave almost equivalent results. Similarly, Gerhäusser et al.[9] found that, for discriminating several benzodiazepines with the WC method, the use of derivative spectra enhanced the selectivity of the library dramatically.

Wavelength selection can also be considered as a tool to optimise the method. Kramer[10] et al. showed that the use of pretreatments such as the first derivative and MSC, combined with wavelength selection, improved the chemometric algorithms of principal component analysis (PCA) and of soft independent modelling of class analogy (SIMCA) for discriminating among several cellulose ethers. For differentiation of two types of cellulose with PCA, the use of the selected spectral range from 1400 nm to1500 nm was superior to the use of the whole NIR spectral range. The influence of differences in humidity can be eliminated by leaving out the water absorbance region from 1880 nm to 2100 nm, thus improving correct classification (i.e. the technique using SIMCA of the first derivative, MSC-treated spectra). They concluded that wavelength selection and careful spectral pretreatment are important for reliably classifying pharmaceuticals with the combined use of NIRS and chemometric algorithms.

Gerhäusser et al. observed that exclusion of wavelengths (i.e. wavelength ranges related to water) did not improve the method of maximum wavelength distance (MWD) or WC on second-derivative spectra[9]. Ulmschneider and Penigault[11] also included wavelength selection in the methods they developed. In all the other studies we reviewed, no wavelength selection was applied.

The use of a wide NIR spectral range of 1000 nm - 2500 nm is most common for verifying identity.

3.1.2 Chemometrics

The WC, MWD, and PCA chemometric algorithms are the methods most commonly used for identity testing (Table 3.1). WC represents the correlation between two spectra, which is based on the sum of the individual correlation of absorbances of each included wavelength. MWD is the maximum value of the standard deviation when an unknown spectrum is compared, on each included wavelength, to the mean spectrum of the training set. Both WC and MWD concern fixed mathematical equations.

PCA employs a technique in which the principal components of a large set of data are defined. In this case, the data include all values at each included wavelength for each spectrum. The principal components should cover the greatest variation within this data set. The information in each spectrum is reduced to one data point on the PC plot. Each PCA model is unique being the result of its development and the applied software.

Other methods are (1) the Mahalanobis distance (MD), which involves a PCA technique, (2) the spectral match value (SMV), which is the cosine value between the sample spectrum and the reference spectrum both regarded as a vector, and (3) the conformity index (CI), which is comparable to MWD. SIMCA, based on PCA, and BCAP are software-related classification techniques.

Gemperline[7] compared three chemometric algorithms, without pretreatment, and found that overall, when small training sets were used, the MWD method gave better classification results than the MD and SIMCA methods.

3.1.3 Combinations of pretreatments and chemometrics

Several combinations of pretreatment, including wavelength selection and chemometric algorithms, are possible and have been examined. Candolfi et al.[12] examined all the combinations of four pretreatments and four chemometric algorithms. MWD combined with detrending or SNV as a pretreatment proved to be the best method. The WC algorithm was not included in these tests. The same study shows that, for SIMCA and MWD, derivative spectra gave worse results than the raw spectra. One study[9] concluded that the classification method of the correlation coefficient preceded by PCA, on the second-derivative spectra, fulfils all the requirements of a suitable pattern recognition method in that it yields reliable results even when the training set is relatively small. In this case, correlation coefficients are calculated between the PCA score of the unknown spectrum and the mean PCA score of each product included in the reference library. A training set consists of the set of spectra of samples in the reference library that is relevant to the same substance or property.

One optimal combination cannot be presented. Good results and experiences are described for WC or PCA of first or second derivatives[8–15] and MWD with pretreatments different from the derivative treatments[2,7,12].

3.2 Thresholds

The thresholds, which are set in the process of calibration, are decisive for the results of the sample analysis.

A threshold correlation of 0.95 is commonly used for WC on the second-derivative spectra[9,14]. Higher thresholds are also possible for detecting the presence of impurities in substances, but make the method less robust for batch-to-batch variations within a substance[8]. Gemperline et al.[7] propose using the probability threshold instead of the distance threshold for MWD, MD, and SIMCA because it is less affected by a change in the number of batches included in the calibration set, and by changes in the wavelength range.

3.3 Calibration set

The number and nature of the batches of the calibration set are critical for its representativeness and reliability. The larger the number of batches in its training sets, the more reliable a method will be. However, the choice of these batches is just as important.

A method can only identify samples for which it has been trained. Therefore, the information on the products should include the quality and variability of the physical characteristics. Note that the number of batches included in the calibration set can be kept small for any given substance or test property[14]. If many variations are possible, larger amounts should be included. For example, if a substance is obtained from several suppliers, although the substance always meets the set specifications, several variations in properties can still occur (e.g. unspecified properties like particle size distribution, density of the powder, or the moisture content). Then, it is likely that batches from all these suppliers need to be included in the training set to yield a practically applicable method. The training set should include examples from all expected sources of spectral variability[9].

When spectra are collected over several months, instrument-dependent sources of variance, such as the instrument stability over time, are also included in the calibration[12].

If batches from various suppliers are recorded with different optical devices and under varying conditions, then these aspects can be included in the calibration, and these aspects of variance can be covered. If it is properly validated, the method can then be considered robust for these aspects. Many other aspects can also be included in the calibration (e.g. density of the sample, water content, purity profile, and variation in packaging material). It is common usage that all batches included should at least meet the conventional specifications for the substances. In most cases, these are the specifications of the Ph Eur[2,11,13,14].

Candolfi et al.[2] applied training sets composed of a minimum of 15 batches (MWD, SIMCA, and MD).

One study showed that, when applied to the raw spectra, MWD is less sensitive for small training sets than PCA or SIMCA[6]. Where it is important to discriminate closely related substances, better performance can be obtained by using training sets with the same number of samples. The results indicate that fewer identification errors occur when large training sets are used. Another study shows that, when the second derivative is used as a pretreatment, the training sets required for calibrating a library by WC tend to be smaller than when MWD is used. However, at least three to four batches should be included in the training set[9]. For differentiation of several cellulose ethers and celluloses with PCA or SIMCA, Kramer et al[10] used larger calibration sets, i.e. 5 to 35 batches.

It appears that WC is suitable for verifying the chemical identity even with small training sets.

3.4 Validation

According to the ICH guidelines on validation[16,17], a method for testing identity should be validated for specificity and robustness.

3.4.1 Specificity

We note that NIRS methods for specificity have been validated on three levels.

1. Internal validation; all batches included in the calibration set should be identified unequivocally.
2. External validation with ‘identities’; the developed method is challenged with independent batches (batches not included in the calibration set) of the substances that are included in the calibration.

For this purpose, a distinction should be made between calibration batches and validation batches. To develop NIRS calibrations with good discriminative power, the use of the combination of a calibration stage followed by an independent validation stage it is very important[15]. It is considered incorrect to challenge the method with calibration batches only, because these batches are not independent of the method. The use of a separate calibration set and an independent validation or test set is common.

3. External validation with ‘nonidentities’; the developed method is challenged with substances that are not included in the calibration.

In three studies, this external validation set was composed of a limited number (a maximum of ten) of closely related substances or batches that were rejected due to the presence of impurities, deviating particle size, or moisture content[4,7,8].

In one study, the method was only challenged with two substances with little or no structural similarity to the library products. The article concerned remarks that, in order to be able to assess the danger of incorrect acceptances, it is necessary to include substances that exhibit great structural similarity to the products included in the application[9]. Plugge et al.[4] used an external validation set composed of nine very closely related cellulose derivatives for the identification of microcrystalline cellulose. A set composed of all the nine β-lactam compounds circulating in the quality control laboratory was used for testing the identity of ampicillin trihydrate.

Gemperline et al.[7] have challenged their methods with samples that do not meet product specifications and samples adulterated with low levels of contamination. They found that MWD was less suitable in detecting these samples than MD or SIMCA. The latter two could detect contamination of less than 0.5% of sulfanilic acid in sulfamethoxazole substance.

We saw little concern in the literature about this part of the external validation. The methods have not been challenged with ‘nonidentities’, or only with a limited number of them.

3.4.2 Robustness

Yoon et al.[8] showed that their method was robust to small changes in the humidity, path length, wavelength error, and moisture content of the samples. In another study, they investigated the effects of sample presentation on NIR reflectance spectra. The spectral distortions resulting from variations in cup diameter, sample thickness, and cup material were shown to alter the values of two commonly used identification algorithms, correlation coefficient (> 0.95) and maximum distance (< 3.0 standard deviation distance), significantly, sufficiently to cause misidentifications. A sample thickness of 10 mm or more was found to be adequate for most pharmaceutical excipients. The method of packing, i.e. tapping or just pouring, was also important[18]. It is clear that the robustness of a NIRS method should be

known or investigated and that verifications alone are insufficient for NIRS for proving unchanged reliability after a relevant change to the instrumentation performance.

The inclusion of more than one sample of one batch will not contribute to the representativeness of the calibration set. Testing more than one sample of each batch could, however, increase the robustness of the method for testing variations such as the homogeneity and packaging of the sample. No studies about this issue were found.

3.5 Reliability

In several studies, the reliability of the application was evaluated by determining α and β errors[7,12]. α Errors are incorrect rejections, and β errors are incorrect acceptances. β Errors are, of course, not acceptable in the pharmaceutical industry.

The selected NIRS method must discriminate well enough to eliminate β errors. It should not discriminate too much (i.e. be too robust) so that samples of new batches that have slightly different spectra are still considered as belonging to one class. α And β errors only give an indication of the quality of the calibration. It should be understood that these α and β values are very dependent on how many batches are included in the validation set and on the 'nonidentities' included in the validation set.

3.6 Change control

When a NIRS application has been developed and subsequently used, changes, intended or accidental, will occur. The impact of such changes on the reliability of the application should be controlled. We can distinguish between changes in the instrumentation and changes in the applied method.

A monograph on NIRS is included in the Ph Eur[6]. Performance verifications of the apparatus are well defined in this monograph. In applications for marketing authorisation, reference can be made to this Monograph for this aspect. Such performance verifications are also discussed in the published in-process revision of the United States Pharmacopeia (USP) monograph *<1119> Near-infrared spectrophotometry*[19].

Some potential changes can be included in the calibration or could be defined as of little effect as result of testing the robustness. If an application is developed, it should be clearly stated which aspects are included in the calibration, which aspects were shown not to

affect the application (validation for robustness), and which aspects need additional testing to confirm unchanged reliability of the application after such a change.

Little about change control is mentioned in the literature, including the Ph Eur and the USP. The Ph Eur only states that ‘the selectivity must be challenged on a regular basis to ensure ongoing validity of the database; this is especially necessary after any major change in a substance, for example: change of supplier or in the manufacturing process of the material’[6]. Nothing is said about the validation after a change of the chemometric algorithm and the pretreatment, the thresholds, or the composition of the calibration set.

Model updating consists of incorporating new sources of variance in the classification model in order to make it more robust and maintain its applicability. This model updating implies widening thresholds and thus possibly more incorrect acceptances. Candolfi et al.[2] showed that the univariate method MWD is less sensitive to this danger than the multivariate methods SIMCA and MD, applied in the principal component phase. It can be assumed that model updating is more straightforward for univariate methods like WC and MWD than for multivariate, PCA-based methods.

3.7 Transferability

Calibrations were shown to be transferable to other spectrometers[11,13–15]. All these studies concerned spectrometers (and software) of the same brand and type, and PCA or WC on first or second-derivative spectra. Calibrations that are based on small differences may require the mixing of spectra recorded on different spectrometers in order to increase the ruggedness.

Calibrations that included spectra recorded on three different spectrometers of the same type showed that they provide an application that is robust in this aspect and that is transferable. Ulmschneider et al.[15] used this method to create a transferable library, mainly of benzodiazepines. The data are now part of a commercially available NIRS library. This library could be transferred to any spectrophotometer of the same brand and type without the need of any transferability tools or correction algorithms.

They state that the concept of a competence centre that builds up, maintains, and forwards the NIRS calibrations to users is now realisable, provided the spectrometers used are of the same type. Furthermore, this kind of calibration can be used as a starter library for companies just beginning to use NIRS for identifying incoming goods at the warehouse.

One study showed promising results of transferability even among three spectrometers from different suppliers[8]. The calibration included spectra recorded on all three

types of spectrometers. This study concerned transflectance testing in solvents. It can be expected that transferability of such a method is less complicated than that of reflectance methods with solids.

We know of no other studies of transferability between different types of spectrometers. Transferability is expected to be less complicated for spectrometers of the same brand and type.

3.8 Conclusions from the literature review

One optimal combination of pretreatment and chemometric algorithm cannot be presented. Wavelength selection and careful spectral pretreatment are important for the reliable classification of pharmaceuticals by NIRS. A wide NIR spectral range of 1000 nm - 2500 nm is most commonly used.

Good results and experience are described for the chemometric algorithms of wavelength correlation (WC) and principal component analysis (PCA) on derivative spectra (first or second). Maximum wavelength distance (MWD), with pretreatments different from applying derivatives, is also commonly used. While WC and MWD are standardised chemometric algorithms with a fixed mathematical equation, each PCA model is unique because its characteristics are determined by the individual using the software that develops it. A threshold correlation greater than 0.95 is commonly used for WC on second-derivative NIR spectra.

Fewer identification errors occur when larger training sets are used. It seems that WC is suitable for verifying the chemical identity, even for small training sets. All batches included in the calibration set should at least meet the conventional specifications of the substances. In most cases, these are specifications of the Ph Eur. The calibration set should include examples of all expected sources of spectral variability. Many aspects can be included in the calibration (e.g. density of the sample, water content, purity profile, and variation in packaging material).

The literature says little about validation for specificity of substances that are not included in the method (‘nonidentities’). The methods have been challenged with a limited number of such substances or none at all. An appropriate method for the quantification of the reliability has not been described. It is clear that the robustness of a NIRS application should be known or investigated and performance verifications alone are deemed insufficient for confirming unchanged reliability after a relevant change of the instrumentation.

It can be assumed that model updating is more straightforward for univariate methods like WC and MWD than for multivariate, PCA-based methods. Little information was found on change control.

Calibration sets can be transferred to others spectrometers, notably spectrometers of the same brand and type. By this, the concept of a competence center building up and maintaining the NIRS calibrations and forwarding them to users is yet realizable, provided the use of spectrometers of the same brand and type.

4 Tentative minimum requirements

The experience and knowledge of the Organon quality laboratory, the results of the literature review, and the regulatory experience of the LGO were the input for the cooperative formulation of a proposal for minimum requirements defined in the draft technical report *Identification of active substances and excipients with NIRS*. At that time, these requirements were considered as tentative minimum requirements, and the document was simply called a draft technical report (Appendix 1).

We have the following remarks about these tentative minimum requirements.

- According to the ICH guidelines on validation[16,17], a method for testing identity should be validated for specificity and robustness. Concerning specificity, the Note for Guidance on setting specifications states that identification testing should optimally be able to discriminate between compounds of closely related structure that are likely to be present[20]. The Technical Guide for the elaboration of monographs, an official publication of the Ph Eur organisation European Directorate for the Quality of Medicines (EDQM) gives guidance for the elaboration of monographs. It states that 'the specificity of the identification should be such that active substances and excipients exhibiting similar structures are distinguished' and also that 'they do not require more experimental effort than necessary for differentiating the substance in question from the other pharmaceutical substances available in commerce'. It is also indicated that the identification must always be validated[21]. The Ph Eur itself indicates that, if a pharmacopoeial method is replaced by an alternative method, it should be proven that the alternative method is at least equal to the pharmacopoeial method[22]. This could be shown by cross-validation. However, because the relation between NIR spectra and substance properties, including the chemical structure, is not clear, validation for specificity of a NIRS application cannot be addressed in the same way as that for other identification methods. A different approach should be considered. A possible approach is composing the validation set with substances that are likely to be present, including name- and structure analogues, instead of only structure analogues that are likely to be present.

- The subsequent use of more than one chemometric algorithm (multiple qualitative data analyses) has several advantages. For verifying identity with NIRS, this implies that the

first chemometric algorithm yields classes of substances with, concerning this first chemometric algorithm, similar NIR spectral characteristics. The second chemometric algorithm differentiates substances in one class from one another, so these substances are verified unequivocally. It is also possible to use the same chemometric algorithm twice. Then the threshold used in the second step (thus within a class of substances) is tighter than in the first step.

- The maintenance of the NIRS application should be clearly defined. For any possible change in the method, in the instrumentation, the applied chemometrics, pretreatments, and composition of the library, one should ask what effect this change could have on the performance of the whole method, and so on the reliability of the results. The kind of verifications and/or validations necessary to prove this maintenance of reliability should be defined. The same aspects are relevant if the method is to be transferred to other equipment.

5 Development of a NIRS analytical application

5.1 Introduction

The aim of this part of the project was to develop and validate a NIRS application for verifying the identity of pharmaceutical substances in a pharmaceutical setting within the tentative minimum requirements that are defined in the '*Draft technical report - identification of active substances and excipients with NIRS*. We emphasise that it was certainly not the intention to develop an optimal application; an evaluation of the defined tentative minimum requirements should be based on the experience with an application that, although in compliance with these tentative minimum requirements, is as far from optimal as possible but still permissible.

One NIRS application composed of several NIRS methods was developed to verify the identity of 12 chosen pharmaceutical substances. These substances were prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, glycerol 85%, macrogol 300, Precirol, Lubritab, and three physical forms of paracetamol, namely 45µm, 180µm, and crystalline.

The substances Precirol and Lubritab were added by special request of Magnafarma because discriminating between these two substances with other techniques, including MIR-spectroscopy, was complicated. Precirol is an emulsifier that consists of atomised glycerol palmitostearate made of mono-, di- and triglycerides of saturated fatty acids. Lubritab is a dry powder made from hydrogenated refined cottonseed oil. It complies with the Monographs Hydrogenated Oil BP and Hydrogenated Vegetable Oil, Type 1, NF.

See Appendix 3 for a description of the development and validation of the NIRS application.

5.2 The NIRS application

As result of the development, the NIRS methods chosen were defined as follows:

Verification of identity of:

- 1. Precirol and Lubritab

  Wavelength correlation on raw spectra over range 10 000 cm^{-1} – 4000 cm^{-1} with a threshold of 0.98 (Method A)

- 2. Prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, glycerol 85%, and macrogol (300 or 400). (Macrogol 300 cannot be differentiated from macrogol 400 by this NIRS application; an additional test, e.g. on viscosity, is required.)
  Wavelength correlation on second-derivative spectra for the range of 7000 cm^{-1} – 4000 cm^{-1} with a threshold of 0.95 (Method C)

- 3. Paracetamol 45 µm, 180 µm, and crystalline
  Wavelength correlation on second-derivative spectra for a range of 7000 cm^{-1} – 4000 cm^{-1} with a threshold of 0.95 and subsequently SIMCA with models M14, M15, and M16 with a 0.01 critical probability level (Method D)

6 Comparison of NIRS and the conventional methods

The four NIRS methods A, B, C, and D (Appendix 3) were evaluated for their suitability to verify the identity and for robustness. The results of method B are informative only because method B has not been defined as method of choice in the NIRS application. Both instrumental and applied chemometrics were evaluated for robustness.

6.1 Method

The NIRS methods were challenged with 24 samples, received as two sets of 12 samples, which had already been tested for release at the Quality Laboratory of Magnafarma. The first 12 samples were presented with a claimed identity. The second set of 12 samples was presented as coded unknown samples. Magnafarma was invited to include samples that should be rejected in view of their conventional analysis.

The first set of 12 samples included 4 samples from batches that had already been used in the calibration process. These batches were not considered independent, so their results were not used for the evaluation. Two samples were from batches that had already been used in the validation process. The results of these samples were used as additional information. One sample was provided as prednisone from a batch that had already been included in the calibration (batch 12646), but appeared to be cortisone acetate. The second set contained only samples of batches that had not yet been analysed with NIRS.

Robustness was investigated with the samples of Precirol and Lubritab because verifying their identities was considered the most critical of all the included substances. The samples of the ten Precirol calibration batches and the six Lubritab calibration batches were analysed five times with NIRS during a period of 12 months (October 2000 to October 2001).

The NIRS analysis, with methods A, B, C, and D, was carried out as described in Sect. 4 and Appendix 3. For testing robustness, correlation coefficients were determined for the Precirol and Lubritab spectra. PCA was used additionally to obtain an indication for the clustering of data and possible trends in these data during the period of 12 months.

6.2 Results

6.2.1 Verification of identity

The results of testing the first 12 samples with methods A and B are given in Table 6.2.1. The correlation coefficients for Precirol and Lubritab (method A) were larger than 0.98. The claimed identity was therefore accepted. The correlation coefficients were larger than 0.95 (Method B) for all other samples. The correlation coefficients with method C were larger than 0.95 for all samples. The claimed identities of all these samples were therefore accepted except for sample 12646. This sample was identified by both methods C and B as cortisone acetate. The identity of this sample was confirmed with MIR-spectroscopy as cortisone acetate. In view of this, the sample was correctly rejected as prednisone.

Table 6.2.1. Correlation coefficients with methods A, B (raw spectra, range 10 000 cm^{-1} – 4000 cm^{-1}), C (second-derivative spectra, range 7000 cm^{-1} *– 4000 cm^{-1})*

Claimed identity	Sample	Result A/B	Conclusion	Result C	Conclusion
Precirol #	12822	0.9999	+	0.9986	+
Lubritab #	12221	0.9999	+	0.9995	+
Furosemide	14026	0.9999	+	0.9986	+
Tolbutamide	12346	0.9998	+	0.9985	+
Prednisone	12646	0.9996	Cortisone acetate	0.9923	Cortisone acetate
Prednisolone	13872	0.9992	+	0.9969	+
Cortisone acetate	13898	0.9995	+	0.9899	+
Paracetamol 180 µm #	12218	0.9998	+	0.9957	+
Paracetamol 45 µm	10571	0.9998	+	0.9912	+
Paracetamol crystalline	14694	0.9952	+	0.9936	+
Glycerol 85%	13823	0.9999	+	0.9930	+
Macrogol 300 #	11924	0.9947	+	0.9588	+

Conclusion **+** indicates that the result concerns confirmation of the claimed identity.
The samples marked with an **#** are from batches that were already used for the calibration.

Two samples of paracetamol were classified by WC as paracetamol and additionally identified with SIMCA as paracetamol 180 µm (sample 12218) and paracetamol 45 µm (sample 10571); however, with a probability level of 0.001. They were rejected when the defined critical probability level of 0.01 was used. Sample 14694 (claimed as paracetamol crystalline) was also classified by WC as paracetamol, but not identified as one of the three forms included in the method (crystalline, 45 µm, and 180 µm). Therefore all paracetamol samples were rejected when the defined critical probability level of 0.01 was applied.

Inclusion of the spectrum of sample 14694 in the PCA plot of the paracetamol reference samples, which was used for creating the SIMCA models (Appendix 3), showed that this spectrum is clearly outside the clusters of the included forms (Figure 6.2.1). Inspection of the sample under the microscope revealed that it was not homogeneously crystalline. The sample clearly deviated from the paracetamol crystalline reference samples. On basis of the PCA plot and the microscopic examination, the samples of paracetamol 45 µm and 180 µm were not found to differ from reference samples. Moreover, the sample of 180 µm came from a batch that was included in the training set.

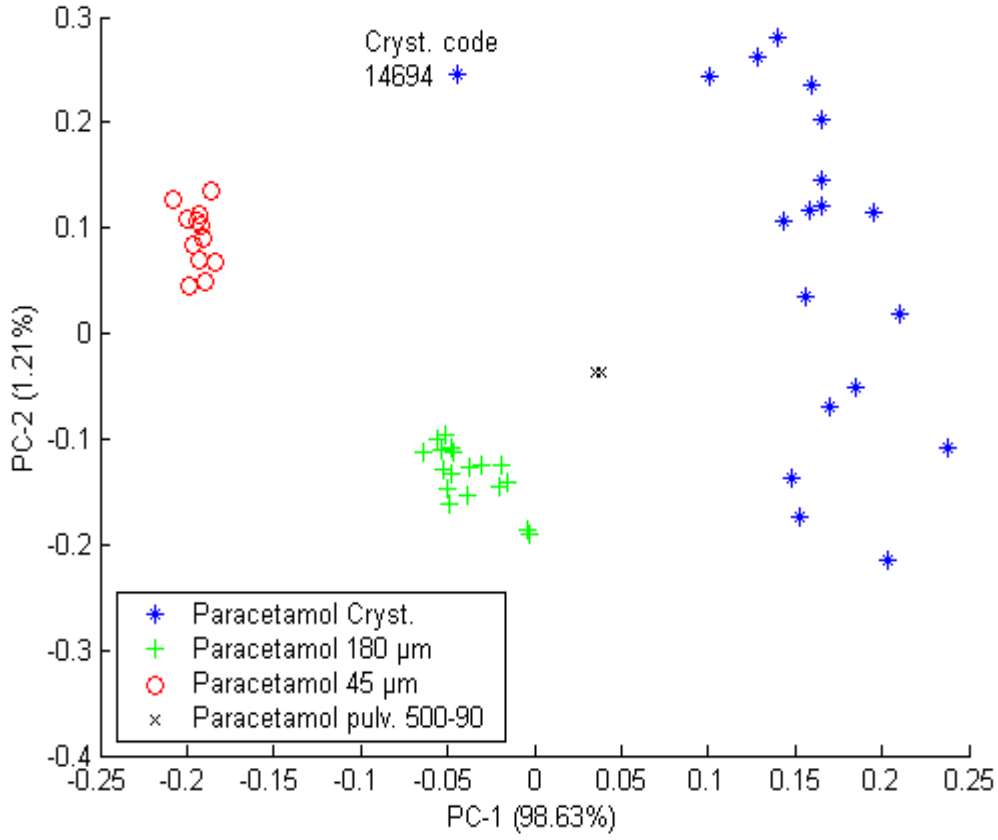


Figure 6.2.1. PCA plot of paracetamol 45 µm, 180 µm, crystalline and 500-90; PC1/PC2 of second-derivative spectra, range 10 000 cm^{-1} *– 4000* cm^{-1}

The three paracetamol samples were all approved for release at the quality laboratory as the defined form. Three issues might be relevant:

1. The sampling and treatment during storage and transport differed from that of the samples of the reference batches.
2. The samples were borderline batches with respect to the property particle size.

3. Recording the spectra of the samples of the reference batches in one row on a certain day and subsequently defining a strict threshold yielded a threshold that was too limited; too little normal variation was included in the calibration.

The rejection of the samples of paracetamol 45 µm and 180 µm was most likely due to points 1 and/or 3, and the rejection of the sample of paracetamol crystalline could be due to all three points.

For paracetamol (Method D), we concluded that the spectra in the reference library did not sufficiently represent the normal variation to be expected. This could have to do with variation in sampling, treatment and analysis of the sample (robustness of the method), or variation in the particle size of the substance.

The measuring conditions were as reproducible as possible from one sample to the other, and conformed to the Ph Eur monograph *Near infrared spectrophotometry*. Whether the samples were taken reproducibly (full or almost empty container) can be questioned; perhaps some variation in the homogeneity of the samples at the moment of testing could have been relevant. In such a case, additional standard sampling, storage, and/or treatment i.e. homogenisation of the sample before analysis could be considered. It would be preferable not to record all the spectra for the reference library on one day, so that the calibration will contain more normal variation. The second point could be dealt with by including more reference batches in the training set, and preferably some borderline batches.

However, in this specific case, it might also be acceptable to define the threshold (i.e. critical probability level) for the SIMCA method less narrowly, in view of the specification tested and the reference method. The SIMCA method differentiates between differently defined forms of the same chemical substance for which the reference methods are ‘visual inspection’ for paracetamol crystalline and ‘sieve tests’ for the other two forms. Both these methods accept quite some variation within the defined form.

Table 6.2.2. Correlation coefficients for methods A and B (raw spectra/range 10 000 cm^{-1} – 4000 cm^{-1}) and method C (second-derivative spectra/range 7000 cm^{-1} – 4000 cm^{-1})

Sample	Code	Result A/B	Conclusion	Result C	Conclusion
Unknown	10570	0.9997	Paracetamol	0.9981	Paracetamol
Unknown	13669	0.6378	No match	0.1738	No match
Unknown	13759	0.5492	No match	0.2263	No match
Unknown	13885	0.9945	Paracetamol	0.9987	Paracetamol
Unknown	14608	0.9922	Precirol	0.9846	Precirol
Unknown	14619	0.9984	Prednisone	0.9980	Prednisone
Unknown	14695	0.9997	Paracetamol	0.9995	Paracetamol
Unknown	14733	0.9994	Lubritab	0.9968	Lubritab
Unknown	15072	0.1942	No match	0.1561	No match
Unknown	15101	0.9994	Tolbutamide	0.9863	Tolbutamide
Unknown	13845	0.6713	No match	0.2462	No match
Unknown	14719	0.8687	No match	0.7027	No match

The results of the NIRS analysis of the 12 unknown samples are given in Table 6.2.2. Seven samples matched one of the included substances when tested with WC methods A, B, and C. Four samples were identified as Precirol (code 14608) and Lubritab (code 14733) with method A and as prednisone (code 14619) and tolbutamide (code 15101) with method C. Three samples were classified as paracetamol. Two of these three were subsequently identified as paracetamol 45 µm (code 10570) with a probability level of 0.01, and as paracetamol 180 µm (code 14695) with a probability level of 0.001. The third paracetamol sample (code 13885) could not be identified as one of the three included forms.

We found the largest correlation with the spectrum of the paracetamol 500-90 by comparing of the spectrum of sample 13885 with all 46 spectra from the external validation set II. This was confirmed in the PCA plot (Figure 6.2.1.). Because the method was not developed and validated for verifying the identity of paracetamol 500-90, this sample could not be considered as having been identified as such with the developed NIRS application.

The sample of paracetamol 180 µm could only be identified as such by adjusting the probability level. The issue of the necessary adjustment of the probability level has already been discussed.

The other five unknown samples were not identified as one of the substances included in the method. Comparison with the spectra of the substances from the external validation set II indicated that the sample with code 13669 was hydrocortisone acetate, the sample with code 13759 was triamcinolone acetonide, and the sample with code 13845 was propylene

glycol. The correlation coefficients between the spectra of these unknown samples and the spectra of the substances from the validation set were all larger than 0.99.

Two unknown samples could neither be identified nor be related to any other spectrum present. See Table 6.2.3 for the results.

Table 6.2.3. Correlation coefficients with method A and B (raw spectra/range 10 000 cm^{-1} – 4000 cm^{-1}) and method C (second-derivative spectra/range 7000 cm^{-1} – 4000 cm^{-1})

Sample	Code	Result A/B	Conclusion	Result C	Conclusion
Unknown	13669	0.9995	Hydrocortisone acetate	0.9942	Hydrocortisone acetate
Unknown	13759	0.9992	Triamcinolone acetonide	0.9983	Triamcinolone acetonide
Unknown	15072	0.3678	No match	0.1823	No match
Unknown	13845	0.9995	Propylene glycol	0.9889	Propylene glycol
Unknown	14719	0.6341	No match	0.4630	No match

The quality laboratory of Magnafarma, the supplier of the samples, confirmed that the identification of the six samples with the NIRS application was the same as the Magnafarma identification It also confirmed that sample14695 indeed contained paracetamol 180 µm, and that the three samples that were correctly found identical to substances included in the validation set. The two samples that did not match any spectrum in the library were miconazole (code 15072) and sorbitol 70% (code 14719). These samples were correctly rejected.

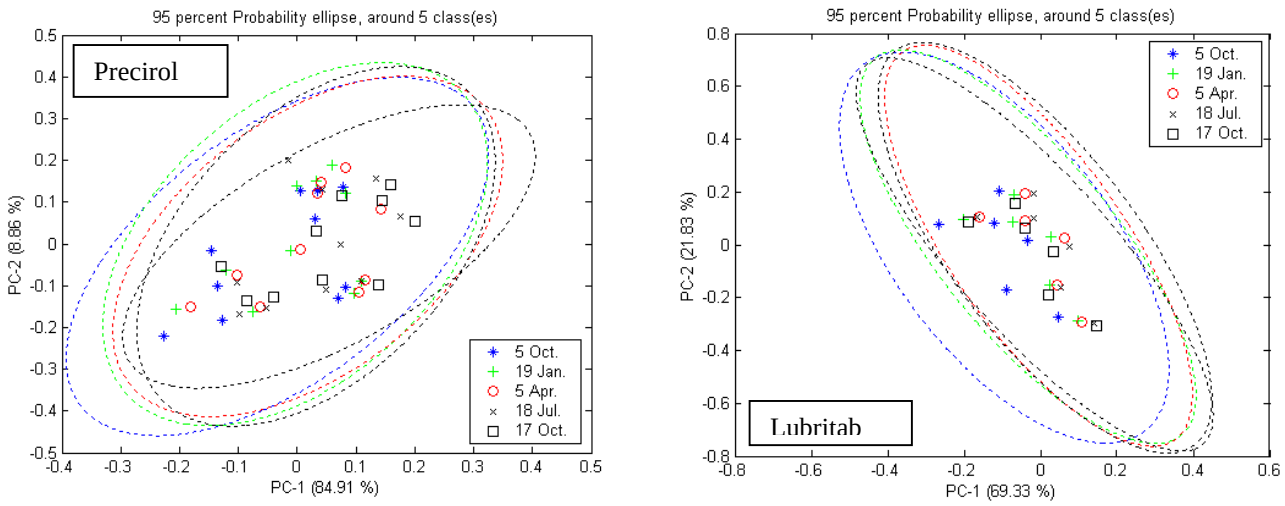
6.2.2 Robustness

Mean correlation coefficients were calculated on raw spectra with a range of 10 000 cm^{-1} – 4000 cm^{-1} (method A) and second-derivative spectra with range 7000 cm^{-1} – 4000 cm^{-1} (as in method C) of Precirol and Lubritab. These spectra were recorded at five times in a period of 12 months. The spectra of the last four times were compared to the spectra recorded at the first time (October 2000). The results are given in Table 6.2.4.

Table 6.2.4. Mean correlation, standard deviation and range in NIR spectra of 10 samples Precirol and six samples Lubritab, analysed at four times between January and October 2001, and compared to the spectra recorded in October 2000 (n = number of spectra)

Precirol		**Raw data 10 000 cm^{-1}-4000 cm^{-1}**			**Second derivative 7000 cm^{-1}-4000 cm^{-1}**		
Date	N	Mean correlation	Standard deviation	Range	Mean correlation	Standard deviation	Range
19 Jan	10	0.9996	0.0002	0.0006	0.9937	0.0028	0.0078
5 Apr	10	0.9996	0.0002	0.0006	0.9927	0.0025	0.0069
18 Jul	10	0.9993	0.0003	0.0009	0.9917	0.0024	0.0087
17 Oct	10	0.9993	0.0003	0.0011	0.9884	0.0036	0.0131
Lubritab							
19 Jan	6	0.9996	0.0002	0.0005	0.9966	0.0030	0.0081
5 Apr	6	0.9996	0.0002	0.0005	0.9964	0.0026	0.0069
18 Jul	6	0.9995	0.0004	0.0009	0.9959	0.0028	0.0076
17 Oct	6	0.9995	0.0004	0.0010	0.9905	0.0034	0.0098

The results from the raw spectra show that there was no difference in mean correlation, standard deviation, and range among the four series of spectra. The mean correlation of the second-derivative spectra for Precirol decreases to 0.9884 and for Lubritab to 0.9905 in the 12 months. No explanation was found for this decrease. The differences in the mean correlation were all very small relative to the distance to the threshold of 0.95 for method C (second-derivative spectra with range 7000 cm^{-1} – 4000 cm^{-1}). Likewise, for method A, the differences in mean correlation (up to 0.0007) were very small relative to the threshold of 0.98.



Figures 6.2.2 and 6.2.3. PCA plots (PC1/PC2) of second-derivative spectra (7000 cm^{-1} -4000 cm^{-1}) of Precirol and Lubritab recorded at five times from October 2000 to October 2001 and the related 95% confidence intervals

PCA was conducted with the 50 Precirol and 30 Lubritab second-derivative spectra with a range of 7000 cm^{-1} – 4000 cm^{-1}. The PCA plots (PC1 versus PC2) of both substances are given in Figures 6.2.2 and 6.2.3. The 95 % confidence intervals were calculated for each time in the analysis. The variance within the spectra recorded at the same time was larger than the variance within the spectra of the same sample recorded at the five different times. The variance over time within the (five) spectra of a sample was very small. The 95 % confidence intervals overlap. This means that no difference could be seen among the data collected during the 12 months.

It was concluded from these results that the NIRS methods A and C are sufficiently robust in time.

The samples of Precirol and Lubritab are suitable for a change control test for the WC methods A and C because the variation in the mean correlation in 12 months is small relative to the thresholds of methods A en C (0.98 and 0.95).

The suitability of SIMCA method D as a change control test was not calculated. A change control test for the developed SIMCA method should concern two substances which discrimination is most critical for that method. Because this method has only been used for to analyse paracetamol, different forms of paracetamol should be considered for this test.

6.3 Conclusions regarding performance

The identities of 8 of the first 12 samples were verified correctly with the NIRS application. The three samples of paracetamol were correctly identified as paracetamol with WC, but could not be identified as any of the included forms with a critical probability threshold of 0.01. The 12th sample was correctly rejected.

Eleven unknown samples of the second set were all correctly identified as substances included in the calibration or were correctly rejected. The critical probability level had to be adjusted to 0.001 for acceptance of the paracetamol 180 µm sample.

For the SIMCA method, it was concluded that the spectra in the reference library did not sufficiently represent the normal expected variation. This may be due to variation in the sampling, treatment and analysis of the sample (robustness of the method), or variation in the particle size of the substance. The method rejected samples incorrectly. However, in this

case, it is acceptable to define the threshold (critical probability level) for the SIMCA method less narrowly, in view of the test specification and the reference method.

For all other identifications, the NIRS application performed well and verified the identities of the samples of substances that were included in the application and rejected the samples of substances not included correctly; the NIRS application performed as well as the conventional methods applied in the quality laboratory.

The robustness of the NIRS methods that are based on WC was examined with samples of the closely related substances Precirol and Lubritab. These two substances gave the most critical discrimination with the WC methods. The methods proved to be robust during the 12 months. The changes in the mean correlation during this period were very small relative to the distance to the threshold used in these methods. In view of this, samples of these substances were considered suitable as a change control test for the WC-based methods.

7 Evaluation of the draft technical report

The tentative minimum requirements that are defined in the draft technical report were evaluated on the basis of the results and experience with the development, validation, and use of the NIRS application, and the comparison with the conventional methods. The adjusted technical report is given in Appendix 2.

7.1 Discussion

7.1.1 The introduction of the draft technical report

The instrumentation specifications, performance verifications, and measurements conform to the Ph Eur monograph *Near infrared spectrophotometry*[6]. Good laboratory practice (GLP) requirements were met when applicable. No problems were encountered on these aspects. We concluded that our study did not raise any doubts or questions about the suitability of the prerequisites defined in the Ph Eur monograph.

7.1.2 The scope of the draft technical report

The conventional methods for verifying the identity were the methods applied by Magnafarma at their quality control laboratory. These are the methods of the Ph Eur for chemical identification. Magnafarma used an in-house method for particle size as a conventional method for additional pharmaceutical identification of the three paracetamol forms. The pharmaceutical identities of the other substances were defined only as the chemical identities, and therefore additional methods were irrelevant. No relevant problems were encountered on this issue.

Because discrimination among the homologues of macrogol was not included in the NIRS application, a conventional method for discriminating among them could not be defined as a reference method. This discrimination should be performed additionally to the NIRS application with a conventional method, e.g. a viscosity, density, or refractive index method. There is no reason to question the suitability of the requirements included in this part of the draft technical report.

7.1.3 Method paragraph

The requirements of this part of the draft technical report were all met.

The different homologues of macrogol were classified as one substance as a result of the validation. No additional NIRS method was developed to discriminate this property in additional to the chemical identity. For paracetamol, an additional SIMCA method was added to the WC method to include particle size as a quality aspect of the pharmaceutical identity of the three forms of paracetamol.

The methods developed differ from the method preferred by the draft technical report. Where the draft technical report prefers the combined use of WC and MWD, WC is chosen, with and without pretreatment, and combined with SIMCA. All these methods met the regimen of the draft technical report and were found suitable. The reliability of the application was investigated and, after adjustment of the definition of the tested identities, was considered proven by the validation.

Therefore, the requirements included in this part of the draft technical report were considered suitable. However, in view of the discussion on the rejected paracetamol crystal sample, it is advisable to include a remark about reproducible sampling, treatment, and analysis of the sample, including sample homogeneity.

7.1.4 Reference library paragraph

The issue of the rejected samples of paracetamol confirmed that indeed the spectra included in the reference library should represent the normal expected variation in the spectra. It also showed that spectra for the training set should not all be recorded on one day, so that more normal variation is included in the calibration. It should, however, be remembered that if this is so, the result would be incorrect rejections, in which case the conventional method can be used for retesting. The risk of false acceptances, which are unacceptable in the pharmaceutical industry, is not increased.

7.1.5 Validation paragraph

The sequence of the validation was evaluated as correct. It researched the possibilities and reliability of the method. As a result, the method was adjusted for some aspects. Although many substances were considered, only a limited number were actually tested for external validation. Our study did not reveal any indication that this approach should be questioned. The criteria defined for the composition of this validation set were therefore evaluated as suitable. Because verifying the identity of the 24 samples did not include incorrect

acceptances of samples, the method was considered reliable for its purpose, and therefore the approach of the validation was evaluated as appropriate.
The representativeness of the training set could have been investigated better in the validation if more than one or two batches of each substance or property had been used for external validation I.

Concerning robustness, only the effect of time was investigated. Other aspects that are mentioned in the draft technical report were not examined. Investigation of the influence of nonhomogeneity of the sample should be added to the draft technical report.

7.1.6 Change control paragraph

Suitability of the change control protocol was investigated. One change control test was tested for robustness. The test with the Precirol and Lubritab samples was robust and was considered suitable as change control test for the WC-based methods.

7.2 Conclusions about the technical report

In general, the requirements defined in the draft technical report were found to be suitable and adequate. However, the following remarks should be added to the report:

a. Reproducible sampling, treatment, and analysis of the sample, including homogeneity of the sample, should be considered, when relevant (Appendix 2, point 3).
b. Spectra for the training set should not all be recorded on one day, but over an appropriate period of time, so that more normal variation is included in the calibration (Appendix 2, point 4).
c. Preferably more than one or two batches of each included substance or form are included in the external validation set I (Appendix 2, point 5).
d. The influence of the nonhomogeneity of the sample should be investigated (Appendix 2, point 5).

The adjusted technical report can be found in Appendix 2.

8 Conclusions

8.1 General

Near infrared spectroscopy (NIRS) is a modern technique that is acceptable as a stand-alone method for verifying the identity of pharmaceutical substances. The requirement is a careful construction of the reference library and the development and validation of the application to minimise the chance of accepting samples that should be rejected.

NIRS provides a rapid and environmentally friendly analysis that can be conducted in the production area without the need of highly trained personnel. The actual measurements can be carried out by inserting a so-called probe into the container to be examined or onto the transparent packaging. It is especially suitable for replacing repetitive analyses at a more or less fixed setting, such as single-container verification of the identity of incoming pharmaceutical substances, notably if supplied in many containers.

It is expected that the introduction of NIRS in the pharmaceutical industry for verifying the identity of pharmaceutical substances will increase the compliance to the obligation to verify the identity of every single incoming container and hence decrease the chance of accidents due to mixing up substances that might affect public health.

The technique not only responds to the molecular structure of the tested substance, it is also sensitive to certain physical properties, such as particle size, water content, and polymorphic form. Control of such properties may be needed as part of verifying the identity of starting materials for specific pharmaceutical dosage forms. For each NIRS application, it should be clearly stated which parameter is verified: only the chemical identity or certain physical properties as well, and whether additional conventional methods should be used for further classification.

Different from most of the Ph Eur methods that control only identity and other quality parameters like expected impurities, NIRS analysis may also indicate the presence of unexpected contamination at macro levels. The sensitivity for contamination was not tested in the practical part of this study.

Different from the current, widely accepted, compendial identification method of mid-infrared spectroscopy (MIR), where identification is based on visually comparing the spectra, identification with NIRS is based on comparing spectra with objective algorithms; hence it is expected to be more reliable.

8.2 Chemometric method

Several combinations of chemometrics and pretreatments can be used in a NIRS application for verifying the identity of substances. One optimal combination cannot be presented. Good results and experiences are described for the wavelength correlation (WC) and principal component analysis (PCA) methods of first and second-derivative spectra and the maximum wavelength distance (MWD) method with nonderivative pretreatments. A wide NIR spectral range of 1000 nm – 2500 nm is most commonly used.

The combined use of the chemometric algorithms of WC (for chemical identity) and MWD (for further classification) is preferred for several reasons.

For regulatory reasons, the use of methods based on standard mathematical equations, such as the WC and MWD methods, on the whole NIR range (1000 nm – 2500 nm) are preferred to PCA-based methods since each PCA model is unique. Both the industry and regulators can acquire experience with these preferred methods and gain confidence. Methods with the same standardised chemometrics can more easily be compared.

Moreover, a major advantage of WC is its ability to address a fixed value to the threshold. In SIMCA (a PCA-based method), thresholds are based on building a multivariate probability model and fixing the estimated α error (incorrect rejections). In practice, this method appears to be extremely sensitive to the time span used to collect the spectra of the reference library. This indicates that the difference in size of SIMCA models can be very large, and therefore the difference in β errors (incorrect acceptances) can be large. Thus, building and maintenance demand more effort and expert judgement for SIMCA methods than for WC methods.

For the MWD method, thresholds are based on the variation of responses at individual wavelengths. This implies that the β error in MWD depends on the span of the spectra involved. Since MWD is basically univariate, additional control of this variation will be more straightforward than of SIMCA. MWD was not tested in the practical part of this study.

It can be assumed that model updating is more straightforward for the univariate WC and MWD methods than for PCA-based methods.

Finally, for verifying only the chemical identity, the WC method is suitable even for a small number of calibration batches.

All batches included in the calibration set should at least meet the conventional specifications of the substances involved. In most cases these are the specifications of the Ph Eur. These batches should represent the normal expected variation of the substances

(different suppliers, variation in physical properties, etc.) and the spectra should represent the expected spectral variation (variable sampling and test conditions).

For WC of the second-derivative spectra, a minimum of three batches is required. Validation of the method should demonstrate that the spectra of an acceptable minimum of batches are included in the training set and that these batches and spectra are sufficiently representative.

A threshold correlation greater than 0.95 is commonly used for WC on second-derivative spectra. Chemometric thresholds should be determined during validation of the method.

8.3 Validation

To replace methods of the Ph Eur with NIRS, it should be proven that this alternative method is at least equally as good as the pharmacopoeial method. An appropriate method for quantifying the reliability of identity verification was not found. Therefore, the reliability should be demonstrated during validation. This validation is therefore critical and should be thorough, in compliance with a standard approach.

The validation method presented in the draft technical report has proven acceptable. However, preferably more than one or two batches of each included substance or form are to be included in the external validation set I, and the influence of nonhomogeneity of the sample should be investigated.

For validating specificity and reliability, a NIRS application for verifying identity should be challenged with a rationally composed set of other substances. If justified, it is acceptable to consider a substance as a challenge, but not to actually test it as a member of the validation set.

8.4 Robustness and change control

For NIRS, it should be ascertained that recorded spectra can indeed be compared with spectra recorded earlier. The robustness of a NIRS method should be known or investigated. If a method is developed, it should be clear which aspects have been included in the calibration, which aspects were shown not to affect the application (validation for robustness), and which aspects need additional testing to demonstrate unchanged reliability of the method after a

change of method. This should be reflected in a method-specific protocol on how changes should be dealt with (change control protocol).

For demonstrating unchanged reliability after a relevant change to the instrumentation, performance verifications alone are insufficient for NIRS. Changes regarding the position of the sample on the optical window or the use of another spectrometer are not covered by performance verifications. Therefore, revalidation will be necessary in most cases. A suitable change control test may replace full revalidation in some of these cases. This aspect requires additional examination.

Because the composition of the calibration set is decisive for the method, any change of composition can affect the reliability of the application, and therefore requires full revalidation of the whole method. This is also required when the pretreatment, chemometric algorithm, or threshold is changed.

8.5 Transferability

Calibration sets can be transferred to others spectrometers, notably spectrometers of the same brand and type. Therefore, a competence centre that could build up a library of NIRS calibrations, maintain them, and forwarding them to users, provided that spectrometers of the same brand and type are used, is now realizable. This may make NIRS more available to comparatively small companies.

8.6 Recommendations

The suitability and perhaps even the superiority to conventional identification methods should be proven by further investigations, preferably by additional comparison with the current accepted Ph Eur standard of identification methods, such as mid-infra-red spectroscopy (MIR); if possible, quantified. Moreover, it should be determined to what extend NIRS can be used to detect unexpected contamination in substances at macro levels that will not be detected with most compendial methods.

To improve the usability and reliability of NIRS for verifying the identity of pharmaceutical substances, competent authorities should encourage the development of widely available spectral libraries that can be used in the validation process. Initially, these libraries would be brand or type-specific with regard to the spectrometer. We assume that improved usability will encourage the use of NIRS for this application.

Replacing full revalidations with the so-called change control tests requires additional examination.

Acknowledgements.

The authors thank J. Damm and A. Gerich of Organon, Oss, and R. Bouwman and W. den Doop of Magnafarma Zaandam for sharing their knowledge with us and for their advice, critical remarks, and support.

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Appendix 1. Draft technical report

Identification of active substances and excipients with NIRS

1. Introduction

   This report summarises how a reliable NIRS application for identifying active substances and excipients should be developed, used, and maintained. The requirements for the equipment, the control of instrument performance, and the actual measurements are not discussed in this document. We refer to the European Pharmacopoeia (4th edn) monograph *Near infrared spectrophotometry* (2.2.40) for these aspects, or to *good laboratory practice.*

2. Scope of this document

   This document discusses the identification of active substances and excipients with NIRS as an alternative method in the pharmaceutical industry. This means that the conventional method for identifying a substance, as described in Part IIC-1 or IIC-2 of the chemical pharmaceutical dossier of the product is always maintained and kept up to date in these parts of the dossier and concerning substances should always, if-tested, comply to these conventional identification methods.

   There are two reasons for this:

   - NIRS is used as a secondary method, which means that a NIRS application can only be developed and validated with authenticated batches. Batches should be authenticated with conventional identification methods as described in Part IIC-1 or IIC-2 of the dossier.
   - NIRS analysis can only be done as result of extensive and time-consuming development of the method, including the building of a reference library. Because NIRS analyses are still very equipment dependent, possibilities for transferring these analyses to other equipment are still limited. Consequently, controlling authorities are unable to repeat analyses at control laboratories.

   The monographs of pharmacopoeias define the given identification method as: determination of the chemical identity. In the pharmaceutical industry, identification can also include differentiation of different forms of one chemical substance (e.g. particle size, polymorphic form, and viscosity). In the context of this report, identification means the identification as used in the pharmaceutical industry.

3. Method
   a. Identification of substances with NIRS is based on comparing NIR spectral data of an unknown substance (sample) with spectral data of batches of substances present in a reference library. A conclusion can be drawn about an unknown substance with the use of chemometric algorithms for comparing the data:
      - Pass: the substance is considered identical to a substance present in the reference library.
      - No match: the substance is not considered identical to any substance present in the reference library.
      - Ambiguous: the substance is considered identical to more than one substance present in the reference library.

      In the last case, the application needs to be adapted to be able to identify the substance, or interfering substances should be expelled from the application, or, if possible, they should be classified as one substance.

   b. The application can be adjusted by expanding it with a second, additional method (multiple data analysis). With multiple qualitative data analysis, an unknown substance is first classified by one chemometric method. This class contains substances that have comparable spectral data in the first chemometric method. Then a second, different chemometric method is used for further classification, or the first method is used with more class-specific thresholds. Multiple data analysis increases the reliability by decreasing the number of false-negatives.

   c. The combined use of the two chemometric methods of wavelength correlation (WC) of the second derivative, and maximum wavelength distance (MWD) is considered the method of choice. ‘Smoothing’ may be used as additional pretreatment.
      These two methods combine the comparison of spectral data based on the chemical structure of substances (WC) with the comparison of spectral data based on the physical properties of the substances (MWD).

   d. If other combinations or other methods of pretreatment are used, this should be justified.

e. Chemometric thresholds should be determined during validation of the application. For the chemometric method of WC of the second derivative of the spectrum, a threshold smaller than 0.95 is not acceptable.

f. Reliability of the application should be proven by appropriate validation (point 5).

4. Building a reference library.
   a. The collection of spectra in the database comprises the reference library and other spectra.

   b. Spectra of various batches of each substance (the training sets) for the application should be included in the reference library. These batches should be authenticated with the conventional identification methods, as described in Part IIC-1 or IIC-2 of the dossier. These batches should represent the normal variation of the substance (suppliers, variation in physical properties).

   c. The training set for each substance should contain spectra of at least three different batches. Validation of the application should show that spectra of an acceptable minimum number of batches are included in the training set and that these batches are sufficiently representative.

   d. The composition of the reference library should be listed by batch numbers.

5. Validation

   Validation of the application should consist of:

   A. *Internal validation.* Batches included in the reference library are validated for selectivity to each other. All batches of substances included in the application should be identified unequivocally, with no conflict of substances.

   B. *External validation I.* At least one independent spectrum (one that is not included in the reference library) of each substance included in the application, is tested with the application. Each substance should be identified unequivocally, and the results of these tests give information on the adequacy of the chosen thresholds. After this external validation, the application can be adjusted, if necessary.

Possible adjustments are:

- Change of thresholds
- Removal of substances from the application, or, if possible, classification of substances as one
- Change of pretreatment

After adjustment, the application should be validated again, and this should be repeated until a reliable application is obtained.

C. *External validation II*. All spectra present in the database that are not those of substances included in the application are used for this validation.

The applicant should give reasonable assurance that the application is reliable for its purpose. To this end, he should submit the composition of the external validation set and give, for each substance included in the application, a rational for the composition of this validation set. All existing name and structure analogues should be included in the validation set unless their absence is justified. A justification for the absence of an analogue in the validation set could be based on:

a. The number of analogues included in view of the total number of existing analogues (the validation set should be sufficiently representative for the whole of all existing analogues)
b. The expected NIR spectral characteristics of the analogue
c. The probability of its presence at the pharmaceutical setting
d. Some other justification.

Results of external validation II, with the validation set, should be submitted.

All these substances should give ‘no match’.

In the process of development, use, and maintenance of the NIRS application, NIR spectra of each substance present and/or supplied to the pharmaceutical site should be included in the external validation set. If, for any reason, a different NIR spectrometer is introduced to the pharmaceutical site, every effort should be made to transfer this validation set to the new spectrometer.

D. *Robustness.* Effects of temperature (both of the environment and the sample), humidity, different positions of the sample on the optical window, and different sampling presentation devices should be understood.

6. Change control

Change control can be defined as a protocol containing potential future changes in the method and the actions considered necessary to prove that the reliability of the method will not have diminished after these changes. Two types of changes are considered: changes in method and changes in instrumentation. The minimum requirements for change control are given in Tables 1 and 2.

Table 1. Change control method

Change	Internal validation	External validation I	External validation II
1. Identification method	Yes	Yes	Yes
Identification parameter			
Identification threshold			
Spectra pretreatment			
Chemometric method			
Wavelength range			
4. Reference library			
Addition of substances	Yes	Yes	Yes
Deletion of substances	No	No	No
Addition of batches	Yes	Yes	Yes
Deletion of batches	Yes	Yes	Yes

Table 2. Change control instrumentation

Change	Control of instrument performance*	Change control test
Software	No	Yes
New software		
New version		
Hardware	Yes	Yes
Lamp		
Other optical part		
Electronic parts		
Sample module		
Location instrument		
Instrument		

*Conforms to Ph Eur monograph (2.2.40) and GLP

A change control test compares the spectra of the application before and after the change. The change control test for each method or reference library should be composed of minimally two standard sets of substances the separation of which is most critical for that method or

reference library. If the method does not pass the test after the change, then the method should be fully revalidated (point 5).

Some potential changes can be included in the calibration. Reference spectra, for example, can be recorded with several lamps and on more than one spectrometer. If the method is subsequently validated for robustness to these changes, the change control test need not be done after such a change.

7. Glossary

Authenticate: to qualify a batch as suitable for use as a reference batch

Chemometric algorithm: a mathematical pattern recognition method to compare spectra

External validation set II: set spectra of substances not included in the method; used for validation of the NIRS method for selectivity

Reference library: a database containing all training sets

Training set: a set of spectra of all reference batches of a substance that is included in the method

Appendix 2. Technical report

Identification of active substances and excipients with NIRS

1. Introduction

   This report summarises how a reliable NIRS application for identifying active substances and excipients should be developed, used, and maintained. The requirements for the equipment, the control of instrument performance, and the actual measurements are not discussed in this document. We refer to the European Pharmacopoeia (4th edn) monograph *Near infrared spectrometry* (2.2.40) for these aspects, or to *good laboratory practice.*

2. Scope of this document

   This document discusses the identification of active substances and excipients with NIRS as an alternative method in the pharmaceutical industry. This means that the conventional method for identifying a substance, as in Part IIC-1 or IIC-2 of the chemical pharmaceutical dossier of the product, is always maintained and kept up to date in these parts of the dossier and concerning substances should always, if-tested, comply to these conventional identification methods.

   There are two reasons for this:

   - NIRS is used as a secondary method, which means that a NIRS application can only be developed and validated with authenticated batches. Batches should be authenticated with the conventional methods for identification, as described in Part IIC-1 or IIC-2 of the dossier.
   - NIRS analysis can only be done as result of extensive and time-consuming development of the application, including building a reference library. Because NIRS analyses are still very equipment-dependent, possibilities for transferring these analyses to other equipment are still limited. Consequently, controlling authorities are unable to repeat analyses at control laboratories.

   The monographs of pharmacopoeias define the given identification method as: determination of the chemical identity. In the pharmaceutical industry, identification can also include differentiation of different forms of one chemical substance (e.g. particle size, polymorphic form, and viscosity). In the context of this report, identification means the identification as used in the pharmaceutical industry.

3. Method
   a. Identification of substances with NIRS is based on comparing NIR spectral data of an unknown substance (sample) with spectral data of batches of substances present in a reference library. A conclusion can be drawn about an unknown substance with the use of chemometric algorithms to compare the data:
      - Pass: the substance is considered identical to a substance present in the reference library.
      - No match: the substance is not considered identical to any substance present in the reference library.
      - Ambiguous: the substance is considered identical to more than one substance present in the reference library.

      In the last case, the application needs to be adapted to be able to identify this substance, interfering substances should be removed from the application, or, if possible, they should be classified as one substance.

   b. The application can be adjusted by expanding it with a second, additional method (multiple data analysis). With multiple qualitative data analysis, an unknown substance is first classified by one chemometric method. This class contains substances that have comparable spectral data in the first chemometric method. Then a second, different chemometric method is used for further classification, or the first method is used with more class-specific thresholds. Multiple data analysis increases the reliability by decreasing the false-negatives.

   c. The combined use of the two chemometric methods of wavelength correlation (WC) of the second derivative and maximum wavelength distance (MWD) is considered the method of choice. ‘Smoothing’ may be used as additional pretreatment.
      These two methods combine the comparison of spectral data based on the chemical structure of the substances (WC), with the comparison of spectral data based on the physical properties of the substances (MWD).

   d. If other combinations or other methods of pretreatment are used, this should be justified.

e. Chemometric thresholds should be determined during validation of the application. For the chemometric method of WC of the second derivative of the spectrum, a threshold smaller than 0.95 is not acceptable.

f. Reproducible sampling, treatment, and analysis of the sample, including its homogeneity, should be considered when relevant.

g. Reliability of the method should be proven by appropriate validation (point 5).

4. Building a reference library

a. The collection of spectra in the database comprises the reference library and other spectra.

b. Spectra of various batches of each substance (the training sets) for the application should be included in the reference library. These batches should be authenticated with the conventional identification methods, as described in Part IIC-1 or IIC-2 of the dossier. These batches should represent the normal variation of the substance (suppliers, variation in physical properties).

c. The training set for each substance should contain spectra of at least three different batches. Validation of the application should show that spectra of an acceptable minimum number of batches are included in the training set and that these batches are sufficiently representative.

d. Spectra for the training set should not all be recorded on the same day, but over an appropriate period of time, so that there is more normal variation in the calibration.

e. The composition of the reference library should listed by batch numbers.

5. Validation

Validation of the application should consist of:

A. *Internal validation.* Batches included in the reference library are validated for selectivity to each other. All batches of substances included in the application should be identified unequivocally, with no conflict of substances.

B. *External validation I.* At least one, but preferably more than two, independent spectra (not included in the reference library) of each substance included in the application, are tested with the application. Each substance should be identified unequivocally. The results of these tests give information on the adequacy of the chosen thresholds. After this external validation, the application can be adjusted, if necessary.
Possible adjustments are:
- Change of thresholds
- Removal of substances from the application, or, if possible, classification of the substances as one
- Change of pretreatment.

After adjustment of the application, the method should be validated again, and this should be repeated until a reliable application is obtained.

C. *External validation II*. All spectra present in the database that are not those of substances included in the application are used for this validation.

The applicant should give reasonable assurance that the application is reliable for its purpose. To this end, he should submit the composition of the external validation set and give, for each substance included in the application, a rational for the composition of this validation set. All existing name and structure analogues should be included in the validation set unless their absence is justified. A justification for the absence of an analogue in the validation set could be based on:

a. The number of included analogues in view of the total number of existing analogues (the validation set should be sufficiently representative for the whole of all existing analogues)
b. The expected NIR spectral characteristics of the analogue
c. The probability of its presence in the pharmaceutical setting
d. Some other justification.

Results of external validation II, with the validation set, should be submitted.
All these substances should give a ‘no match’.

In the process of development, use, and maintenance of the NIRS application, the NIR spectra of each substance present and/or supplied to the pharmaceutical site should be included in the external validation set. If for any reason, a different NIR

spectrometer is introduced to the pharmaceutical site, every effort should be made to transfer this validation set to the new spectrometer.

D. *Robustness*. Effects of temperature (both of the environment and of the sample), humidity, different positions of the sample on the optical window, different sampling presentation devices and the nonhomogeneity of the sample should be understood or investigated.

6. Change control

Change control can be defined as a protocol containing potential future changes in the method and the actions considered necessary to prove that the reliability of the method will not have diminished after these changes. Two types of changes are considered: changes in method and changes in instrumentation. The minimum requirements for change control are given in Tables 1 and 2.

Table 1. Change control method

Change	Internal validation	External validation I	External validation II
1. Identification method			
Identification parameter	Yes	Yes	Yes
Identification threshold			
Spectra pretreatment			
Chemometric method			
Wavelength range			
2. Reference library			
Addition of substances	Yes	Yes	Yes
Deletion of substances	No	No	No
Addition of batches	Yes	Yes	Yes
Deletion of batches	Yes	Yes	Yes

Table 2. Change control instrumentation

Change	Control of instrument performance*	Change control test
Software	No	Yes
New software		
New version		
Hardware	Yes	Yes
Lamp		
Other optical part		
Electronic parts		
Sample module		
Location instrument		
Instrument		

*Conforms to Ph Eur monograph (2.2.40) and GLP

A change control test compares the spectra of the application before and after the change. The change control test for each method or reference library should be composed of at least two standard sets of substances the separation of which is most critical for that method or reference library. If the method does not pass the test after the change, then the method should be fully revalidated (point 5).

Some potential changes can be included in the calibration. Reference spectra, for example, can be recorded with several lamps and on more than one spectrometer. If the method is subsequently validated for robustness to these changes, the change control test need not be done after such a change.

7. Glossary

Authenticate: to qualify a batch as suitable for use as a reference batch

Chemometric algorithm: a mathematical pattern recognition method to compare spectra

External validation set II: a set of spectra of substances not included in the application; used for validation of the NIRS application for selectivity

Reference library: database containing all training sets

Training set: a set containing spectra of all reference batches of a substance that is included in the method

Appendix 3. Development and validation of the NIRS application

1 Methods and materials

A model was developed for each substance to be identified with the use of chemometric classification methods. First a calibration was done with the reference library as calibration set and then a validation, with independent batches.

The wavelength correlation (WC) and soft independent modelling of class analogy (SIMCA) methods were used for to classify the pharmaceutical substances. The substances are classified into groups by WC with a threshold of 0.95; some groups can be composed of more than one substance. Substances in one group are further classified by SIMCA, yielding the required pharmaceutical identification as defined beforehand.

Table 1. Substances included

Substance	Batches	Appearance
Precirol	10	Solid
Lubritab	6	Solid
Cortisone acetate	10	Solid
Prednisone	10	Solid
Paracetamol 180 µm	6	Solid
Paracetamol crystalline	10	Solid
Paracetamol 45 µm	4	Solid
Tolbutamide	10	Solid
Furosemide	10	Solid
Prednisolone	10	Solid
Glycerol	10	Liquid
Macrogol 300	10	Liquid

The batches included in the calibration and validation sets had already been released by the Magnafarma quality laboratory, on the basis of the conventional reference methods. Four to ten batches were analysed for each of the 12 substances; a total of 106 samples (Table 1). Each sample corresponded to an unique batch. The substances were solid powders or liquids.

The calibration set was composed of spectra of eight batches chosen at random for each substance. Only five batch samples for Lubritab and paracetamol 180 µm and only three for paracetamol 45 µm were available, yielding a total of 85 reference spectra for the reference library. The spectra of the other batches were used for the validation.

1.1 Calibration and internal validation

For the calibration and internal validation, the spectra included in the calibration were compared. WC was applied to the raw spectra and the second derivative over the whole NIR range (10 000 cm^{-1} – 4000 cm^{-1}) and over a selected range (7000 cm^{-1} – 4000 cm^{-1}). The threshold for a positive identification was 0.95. Substances that showed a correlation greater than 0.95 were classified into the same group. The distance is the difference between the correlation coefficient of a substance and the next closest substance in the reference library. A distance of 0.05 or less implies that discrimination between the two substances is impossible with the applied method and threshold (i.e. 0.95).

Substances in the same group were further classified with SIMCA. A principal component analysis (PCA) was performed and a SIMCA model was created for each substance. Univocal identification was achieved with a critical probability level of 0.01 as the threshold.

Subsequently, we defined how each of the 12 substances could be identified by the WC and SIMCA methods with the defined thresholds.

1.2 External validation I

Validation was first conducted with the spectra of independent batches that had been set aside. The numbers of α errors (incorrect rejections) and β errors (incorrect acceptances) should be minimised. The numbers of errors were determined for each method.

WC was used to correlate each spectrum of the validation set with each spectrum in the reference library. For a positive identification, the result should comply to a threshold defined as greater than 0.95, and the distance to spectra of other substances should be greater than 0.05. SIMCA was applied to all validation spectra of one group, and each identity was defined on the basis of a critical probability level of 0.01.

1.3 External validation II

A set of 45 substances was composed to challenge the application on its capability of rejecting ‘nonidentities’. This validation set was composed in conformance with the draft technical report *Identification of active substances and excipients with NIRS*. All possible name and structure analogues for each substance included in the application were considered, and an appropriate and representative selection was included in the validation set (Appendix 4). The composition of this tested validation set is given in Table 8. All 45 substances should be rejected by the NIRS method.

1.4 NIRS analysis

Spectra were recorded with a Perkin-Elmer Spectrum Identicheck FT-NIR spectrometer over the range of 12 000 cm^{-1} – 3000 cm^{-1}, with an integrating sphere (ICRA) in the upper mode, in conformance to standard operating procedure LOC288. A PbS detector was used. The optical resolution was 16 cm^{-1} for all samples (8 cm^{-1} data point resolution). Spectra were also recorded with 4 cm^{-1} data point resolution for samples of Precirol and Lubritab. For each spectrum, 64 scans were co-added. Teflon was used as a background reference for the solid samples, while a Petri dish with a reflector was used for liquids.

A 1-g sample of each solid powder was tested in a 4-ml glass vial with closure (Alltech). The vial was placed upright upon the eye of the spectrometer, and the spectra were recorded in the diffuse reflection mode (reflectance). A 2-ml sample of each liquid was poured into a Petri dish with a metal reflector (Perkin Elmer), which was placed on the eye of the spectrometer. Spectra were recorded in the transflection mode (transflectance).

1.5 Chemometrics

The wavelength correlation and distance were calculated with the programme COMPARE. The classification with SIMCA using PCA was performed with the program QUANT+. The programmes COMPARE, QUANT+, and the algorithm SIMCA are included in the standard software of the Perkin Elmer instrument.

2 Results

Visual inspection of the spectra indicated that the NIR spectra of the various paracetamol forms (45 µm, 180 µm, and crystalline) were similar except for baseline differences. These baseline differences are due to the differences in particle size distribution of these chemically identical samples.

The spectra of Precirol and Lubritab were almost identical (Figure 1). Small differences were observed in the range of 7000 cm^{-1} – 4500 cm^{-1}.

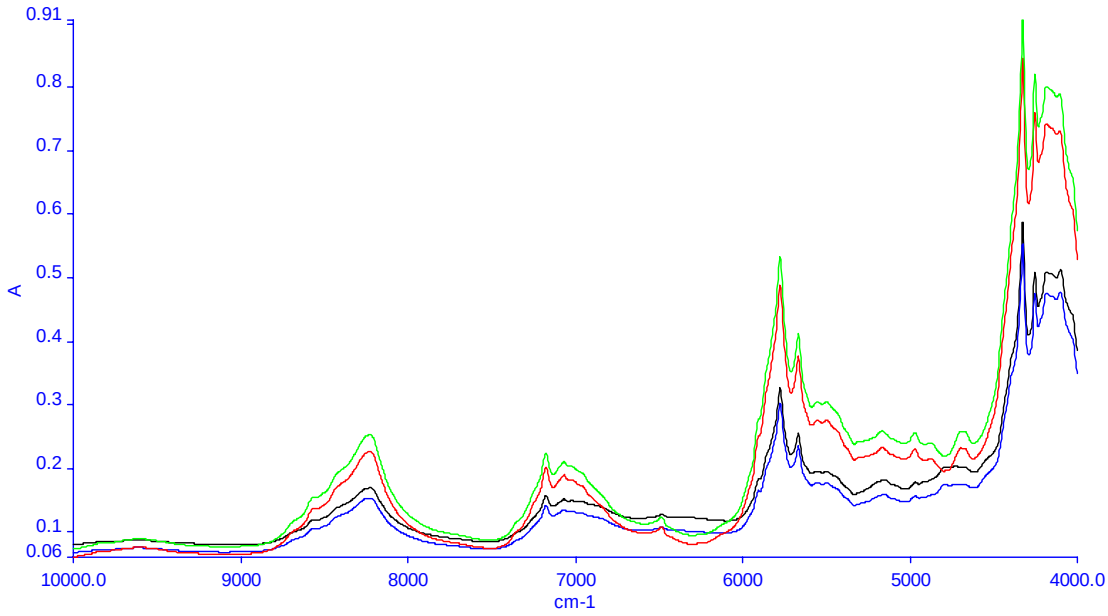


Figure 1. Spectra of Precirol (black, blue) and Lubritab (red, green)

The NIR spectra of prednisone, prednisolone, and cortisone acetate were very different (Figure 2), just like the spectra of furosemide and tolbutamide and the spectra of glycerol 85% and macrogol 300.

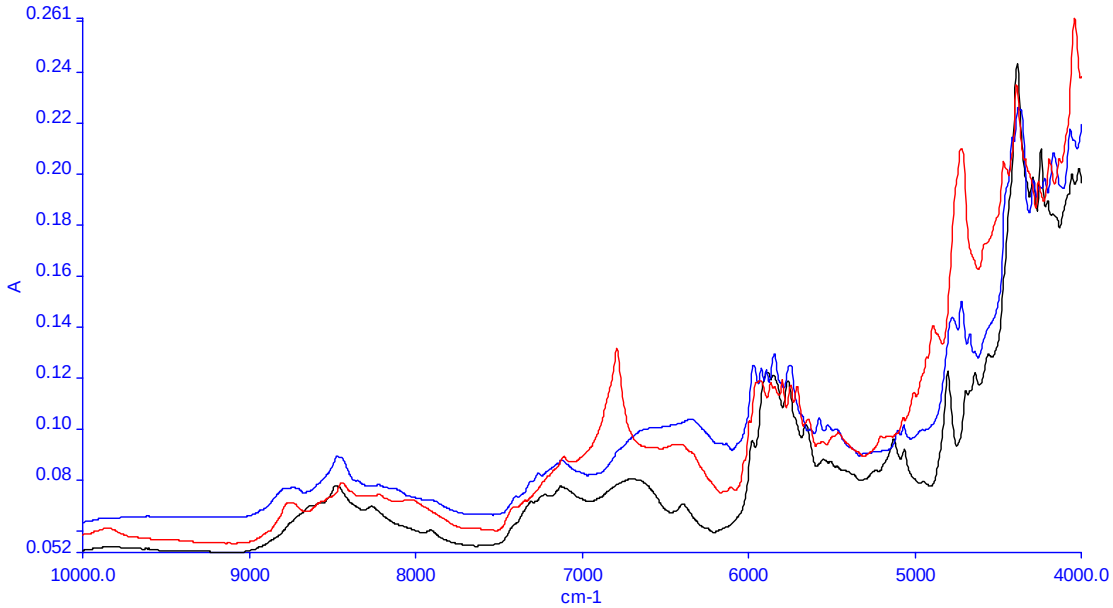


Figure 2. Spectra of prednisone (blue), prednisolone (red) and cortisone acetate (black).

2.1 Calibration and internal validation

Wavelength correlation was applied to the raw spectra over the range 10 000 cm^{-1} – 4000 cm^{-1}; no pretreatment, e.g. applying derivatives, was applied. The results are given in Table 2.

Table 2. Results of wavelength correlation of raw spectra (10 000 cm^{-1} – 4000 cm^{-1})

Substance	N_t	N_c	C_m	S	S_d	D
Precirol	10	8	0.9993	0.0010	0.0004	0.02
Lubritab	6	5	0.9996	0.0007	0.0003	0.02
Cortisone acetate	10	8	0.9987	0.0061	0.0020	0.34
Prednisone	10	8	0.9995	0.0011	0.0004	0.32
Paracetamol 180 µm	6	5	0.9999	0.0001	0.0001	0.00
Paracetamol crystalline	10	8	0.9990	0.0017	0.0006	0.05
Paracetamol 45 µm	4	3	1.0000	0.0001	0.0001	0.00
Tolbutamide	10	8	1.0000	0.0001	0.0001	0.61
Furosemide	10	8	0.9999	0.0001	0.0001	0.79
Prednisolone	10	8	0.9997	0.0009	0.0003	0.53
Glycerol 85%	10	8	0.9999	0.0012	0.0004	0.38
Macrogol 300	10	8	0.9985	0.0084	0.0029	0.42

C_m: mean correlation, D: distance N_c: number of calibration samples, N_t: total number of samples, S: range, S_d: standard deviation,

For all spectra of the reference library, the correlation coefficients to the other spectra of the same substance were larger than 0.95. Table 2 shows that the distance between Precirol and Lubritab is small, i.e. 0.02 or less. The chosen threshold of 0.95 did not separate these substances. This is also true for the various forms of paracetamol. All other substances were unequivocally identified with a threshold of 0.95.

Table 3. Results of wavelength correlation of second-derivative spectra (10 000 cm^{-1} – 4000 cm^{-1})

Substance	N_t	N_c	C_m	S	S_d	D
Precirol	10	8	0.9865	0.0083	0.0034	0.00
Lubritab	6	5	0.9907	0.0049	0.0023	0.00
Cortisone acetate	10	8	0.9788	0.0197	0.0063	0.76
Prednisone	10	8	0.9831	0.0070	0.0021	0.76
Paracetamol 180 µm	6	5	0.9935	0.0031	0.0013	0.01
Paracetamol crystalline	10	8	0.9940	0.0029	0.0011	0.01
Paracetamol 45 µm	4	3	0.9904	0.0016	0.0009	0.00
Tolbutamide	10	8	0.9971	0.0009	0.0003	0.73
Furosemide	10	8	0.9919	0.0025	0.0009	0.82
Prednisolone	10	8	0.9876	0.0061	0.0021	0.87
Glycerol 85 %	10	8	0.8855	0.0160	0.0052	0.58
Macrogol 300	10	8	0.9053	0.0236	0.0089	0.60

C_m: mean correlation, D: distance N_c: number of calibration samples, N_t: total number of samples, S: range, S_d: standard deviation

Table 3 gives the results WC on second-derivative spectra (also in the range of 10 000 cm^{-1} – 4000 cm^{-1}). The correlation ranges and standard deviations of the results from the spectra of the same substance were considerably larger than the results for the algorithm applied to the raw spectra.

The distance in correlation coefficients between the spectra of Precirol and Lubritab was 0.00. This means that no difference could be observed with this method. The distances between the different paracetamol forms were also less than 0.05. It appeared that the mean correlations for glycerol 85% (0.8855) and macrogol 300 (0.9053) were less than the chosen threshold of 0.95. This is due to the large amount of noise relative to the amount of information in the spectral range of 10 000 cm^{-1} – 7000 cm^{-1}. Narrowing the range to 7000 cm^{-1} – 4000 cm^{-1} improved the performance (Table 4).

Table 4. Results of wavelength correlation of second-derivative spectra (7000 cm^{-1} – 4000 cm^{-1})

Substance	N_t	N_c	C_m	S	S_d	D
Precirol	10	8	0.9937	0.0099	0.0031	0.10
Lubritab	6	5	0.9990	0.0002	0.0001	0.09
Paracetamol 180 µm	6	5	0.9997	0.0002	0.0001	0.00
Paracetamol crystalline	10	8	0.9995	0.0003	0.0001	0.00
Paracetamol 45 µm	4	3	0.9996	0.0002	0.0001	0.00
Glycerol 85 %	10	8	0.9912	0.0060	0.0018	0.71
Macrogol 300	10	8	0.9891	0.0111	0.0041	0.71

C_m: mean correlation, D: distance, N_c: number of calibration samples, N_t: total number of samples, S: range, S_d: standard deviation

The distance between Precirol and Lubritab was greater than 0.05 in this case. Both substances could be identified unequivocally by applying both the pretreatments of second-derivative and wavelength selection.

An increase of the spectral resolution from 16 cm^{-1} to 4 cm^{-1} improved the quality of these spectra, but increased the analysis time. This effect was seen for the second-derivative spectra when the whole range (10 000 cm^{-1} – 4000 cm^{-1}) was used. However, the effect was small in the case of the applied wavelength selection (Tables 5 and 6).

Table 5. Precirol: wavelength correlation of spectra with resolutions of 16 cm^{-1} and 4 cm^{-1}

Resolution (cm^{-1})	Pretreatment	Range (cm^{-1})	C_m	S	S_d	D
16	None	10 000-4000	0.9993	0.0010	0.0004	0.02
16	Second-derivative	10 000-4000	0.9865	0.0083	0.0034	0.00
16	Second-derivative	7000-4000	0.9937	0.0099	0.0031	0.10
4	None	10 000-4000	0.9997	0.0006	0.0002	0.03
4	Second-derivative	10 000-4000	0.9328	0.0109	0.0041	0.09
4	Second-derivative	7000-4000	0.9842	0.0038	0.0015	0.11

C_m: mean correlation, D: distance, S: range , S_d: standard deviation

Table 6. Lubritab: wavelength correlation of spectra with resolutions of 16 cm^{-1} and 4 cm^{-1}

Resolution ($cm^{-1)}$)	Pretreatment	Range (cm^{-1})	C_m	S	S_d	D
16	None	10 000-4000	0.9996	0.0007	0.0003	0.02
16	Second-derivative	10 000-4000	0.9907	0.0049	0.0023	0.00
16	Second-derivative	7000-4000	0.9990	0.0002	0.0001	0.09
4	None	10 000-4000	0.9997	0.0005	0.0002	0.02
4	Second-derivative	10 000-4000	0.9449	0.0144	0.0068	0.09
4	Second-derivative	7000-4000	0.9878	0.0117	0.0043	0.10

C_m: mean correlation, D: distance, S: range , S_d: standard deviation

The results of the first classification, i.e. with method WC, can be summarised as follows:

- Two suitable methods for differentiating between Precirol and Lubritab were available: WC of the raw spectra with a threshold of 0.98 and WC on the second-derivative spectra in only the spectral range 7000 cm^{-1} – 4000 cm^{-1} with a threshold of 0.95.
- Two suitable methods were also available for differentiating between glycerol 85% and macrogol 300: WC of the raw spectra with a threshold of 0.95 and WC on the second-derivative spectra in only the spectral range 7000 cm^{-1}– 4000 cm^{-1} with a threshold of 0.95.
- The substances prednisone, prednisolone, cortisone acetate, furosemide, and tolbutamide can be unequivocally identified by WC with a threshold of 0.95. The use of the second-derivative spectra is not necessary for these substances. For comparison, the WC method applied to the second-derivative spectra over the spectral range 7000 cm^{-1}– 4000 cm^{-1} is included in the other validation steps.
- The WC method was incapable of differentiating between the three forms of paracetamol. An additional chemometric method was necessary to identify these substances unequivocally.

At this stage, the methods developed were defined as follows:

Method A: For Precirol and Lubritab: WC of the raw spectra using the spectral range 10 000 cm^{-1} – 4000 cm^{-1} with a threshold of 0.98.

Method B: For prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, glycerol 85%, and macrogol 300: WC of the raw spectra using the spectral range 10 000 cm^{-1}– 4000 cm^{-1} with a threshold of 0.95.

Method C: Alternatively to methods A and B, for the same substances, WC of the second-derivative spectra with a threshold of 0.95 and the selected spectral range of 7000 cm^{-1} – 4000 cm^{-1}.

Method D: For paracetamol 45 µm, 180 µm and crystalline: the subsequent use of WC and another chemometric method.

SIMCA

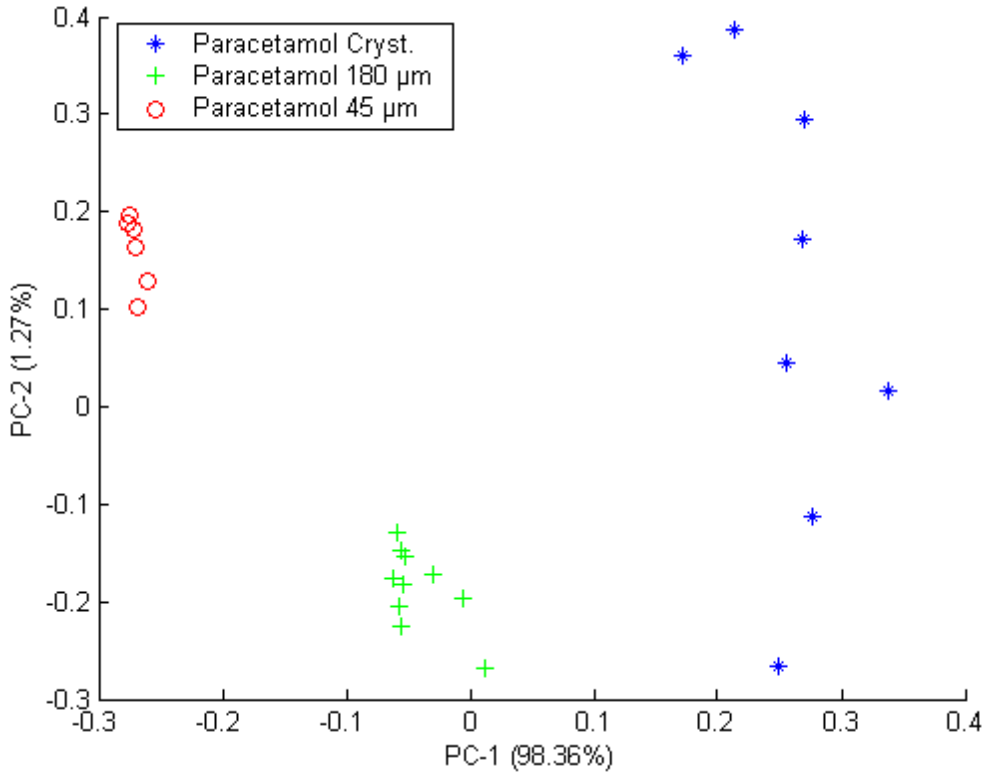
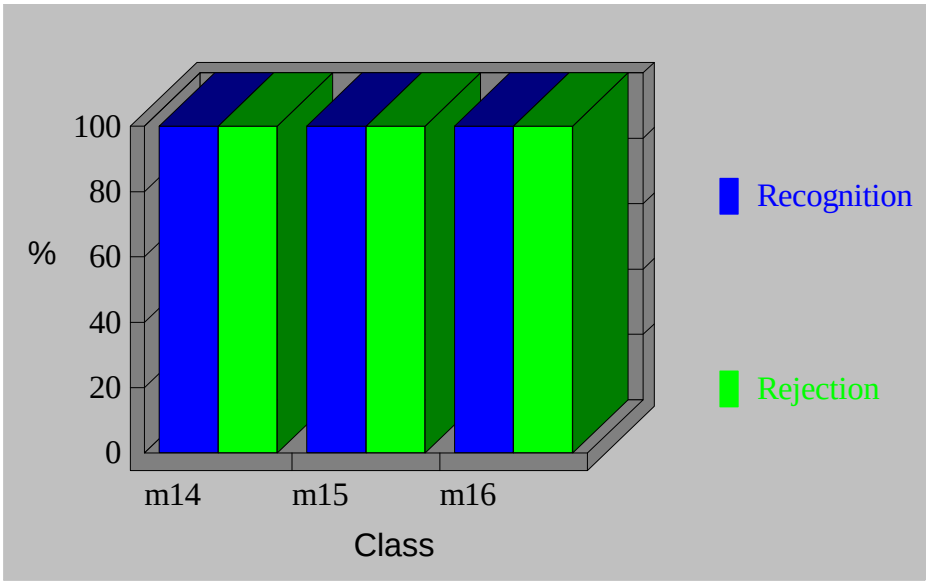


Figure 3. PCA plot of paracetamol 45 µm, 180 µm and crystalline PC1/PC2 of second-derivative spectra (10 000-4000 cm^{-1})

To obtain enough data, i.e. spectra, for principal component analysis (PCA), additional spectra of all the paracetamol samples were recorded. The method was applied to second-derivative spectra in the spectral range of 10 000 cm^{-1} – 4000 cm^{-1}. Multiple scatter correction was used as a pretreatment. Wavelength selection was applied to skip water effects. A correction for water was carried out. The plot is given in Figure 3. It shows three clusters.

On the basis of this PCA plot, a SIMCA model was created for each of the three different forms of paracetamol. The calibration of these three models M14 (paracetamol crystalline), M15 (paracetamol 180 µm), and M16 (paracetamol 45 µm), yields a 100% recognition rate of each model. The diagnostic report of this calibration is given in Figure 4.



Critical probability level: 0.01
Interclass distances

	M14	M15	M16
M14	-	68.27	702.96
M15	-	-	148.73

	Recognition rate (percentages)	Rejection rate
M14	100(8/8)	100(16/16)
M15	100(10/10)	100(14/14)
M16	100(6/6)	100(18/18)

Figure 4. Diagnostic report: M14 (paracetamol crystalline), M15 (paracetamol 180 µm) and M16 (paracetamol 45 µm)

The probability level indicates the chance that a subject presented to the method will be incorrectly rejected. It can be considered as a sphere around the cluster. A larger sphere represents a smaller probability level and hence a smaller chance of incorrect rejection. However, it also implies that more diversity is accepted from a subject still to be accepted as part of the cluster, so it increases the risk of incorrect acceptances. Thus, a lower probability level corresponds with a less strict threshold and method.

2.2. External validation I

The method was challenged with the selected spectra that were set apart from the reference library and therefore were independent spectra.

*Precirol and Lubritab***.** Method A gave correlation coefficients larger than the threshold of 0.98 and distances of 0.02. Method C gave a correlation coefficient greater than 0.99 (much larger than the threshold of 0.95) and distances of 0.08 or greater. The spectra were correctly related to the substances. Although both methods complied, the discrimination was clearer with method C.

Table 7. Results of the external validation I of the WC classification methods

	Method A/B		Method C	
Substance	Correlation	Distance	Correlation	Distance
Precirol	0.9998	0.02	0.9957	0.11
Precirol	0.9998	0.02	0.9965	0.08
Lubritab	0.9998	0.02	0.9989	0.09
Cortisone acetate	0.9998	0.36	0.9972	0.73
Cortisone acetate	0.9996	0.36	0.9958	0.73
Prednisone	0.9997	0.33	0.9975	0.78
Prednisone	0.9998	0.33	0.9978	0.77
Tolbutamide	0.9999	0.62	0.9998	0.73
Tolbutamide	1.0000	0.62	0.9999	0.73
Furosemide	0.9998	0.78	0.9990	0.80
Furosemide	0.9999	0.78	0.9992	0.81
Prednisolone	0.9999	0.54	0.9988	0.86
Prednisolone	1.0000	0.53	0.9991	0.86
Glycerol 85 %	0.9999	0.40	0.9913	0.72
Glycerol 85 %	0.9995	0.39	0.9925	0.71
Macrogol 300	0.9995	0.41	0.9864	0.72
Macrogol 300	0.9986	0.45	0.9829	0.71

Glycerol 85% and macrogol 300. Method B gave correlation coefficients greater than 0.95 and distances greater than 0.39 for both substances. Method C gave correlation coefficients of 0.99 for glycerol 85% and 0.98 for macrogol 300 and a distance larger than 0.71. With both methods, the spectra were correctly related to the substances. Again, although both methods complied, the discrimination was clearer with method C.

Prednisone, prednisolone, cortisone acetate, furosemide and tolbutamide. The spectra of prednisone, prednisolone, cortisone acetate, furosemide and tolbutamide were correctly identified with both methods B and C. In all cases, the distance was much larger than 0.05, viz. for method B, 0.33 or greater, and for method C, 0.73 or greater. Incorrect rejections (α errors) or incorrect acceptances (β errors) did not occur. The defined threshold of 0.95 was appropriate. Both methods complied. The discrimination among the substances was clearer for method C. The results of both methods are given in Table 7.

Paracetamol 45 µm, 180 µm, and crystalline. The validation was conducted with spectra of one independent batch for each form. All three models (M14, M15, and M16) showed 100% recognition of the form tested for and 100% rejection of the two other forms. The three independent validation spectra were correctly identified with a probability level of 0.01.

2.3. External validation II

The methods were challenged with the spectra of the 45 selected substances. All these substances should be rejected as not being one of the 12 substances included in the application. This validation set was composed in conformance to the draft technical report. Appendix 4 lists all the substances that were considered. It also indicates whether the NIRS application was challenged with a recorded spectrum of these substances, and if not, it says why this was not considered necessary. See Table 8 for the recorded substances and the results of this validation.

Method A. No conflicts were observed with Precirol and Lubritab. The correlation coefficients of the 45 selected substances were always less than 0.98. The distance between Precirol and glycerol monostearate was 0.0380. The distance between Lubritab and stearic acid was 0.0719.

Method B. Two matches were observed with method B, i.e. the correlation coefficients of two substances of the 45 were larger than 0.95 compared to a spectrum included in the reference library. Macrogol 400 gave a result 0.9934 compared to macrogol 300, and paracetamol 500-90 gave 0.9943 compared to paracetamol. Only the match of

macrogol 400 concerned an incorrect classification. The match of paracetamol 500-90 with paracetamol is not a conflict because all paracetamol forms may be identified as paracetamol in the first classification step, and discrimination of the several paracetamol forms should take place in the additional SIMCA method.

Method C. Three matches were observed with method C. One of them was again, as expected, paracetamol 500-90. The two other matches were glycerol monostearate (0.9567 compared to Precirol) and macrogol 400 (0.9651 compared to macrogol 300). These matches were considered conflicts. We concluded that the identity of Precirol and Lubritab should be verified with method A and not with method C.

Method D. Paracetamol 500-90 was correctly rejected, and not identified as one of the three forms included in the application.

Table 8. Results of external validation II

Substance		Method A/B		Method C	
		Raw spectra 10 000 cm^{-1}–4000 cm^{-1}		Second-derivative 7000 cm^{-1}–4000 cm^{-1}	
	Code	Correlation coefficient	Closest hit	Correlation coefficient	Closest hit
Paracetamol 500-90	NIR2904	0.9943	Paracetamol	0.9982	Paracetamol
Hydrocortisone	NIR2905	0.5763	Cortisone acetate	0.2956	Prednisolone
Dexamethasone	NIR2906	0.6300	Prednisone	0.2966	Macrogol 300
Glycerol monostearate	NIR2907	0.9620	Precirol	0.9567	Precirol
Acetylsalicylic acid	NIR2908	0.1078	Paracetamol	0.1670	Prednisolone
Ethinyl estradiol	NIR2909	0.2890	Prednisone	0.2226	Furosemide
Mesalazine	NIR2910	0.2284	Paracetamol	0.1769	Cortisone acetate
Hydrocortisone acetate	NIR2911	0.6365	Prednisone	0.1653	Cortisone acetate
Stearic acid pulverised	NIR2912	0.9281	Lubritab	0.8732	Precirol
Prednisolone sodium phosphate	NIR2913	0.6169	Glycerol	0.3229	Prednisolone
Ibuprofen	NIR2914	0.4779	Cortisone acetate	0.1789	Tolbutamide
Testosterone propionate	NIR2915	0.6958	Cortisone acetate	0.2278	Cortisone acetate
Clobetasone butyrate	NIR2916	0.7104	Prednisone	0.2269	Prednisone
Triamcinolone	NIR2917	0.5208	Cortisone acetate	0.1611	Glycerol
Betametasone dipropionate	NIR2918	0.7675	Prednisone	0.2338	Prednisone
Beclometasone dipropionate	NIR2919	0.7461	Prednisone	0.2921	Prednisone
Betametasone valerate	NIR2920	0.6297	Prednisone	0.1874	Prednisone
4-Aminopyridine	NIR2921	0.1253	Cortisone acetate	0.1681	Furosemide
Flumethasone pivalate	NIR2922	0.6526	Prednisone	0.2322	Precirol
Prednisolone hemisuccinate	NIR2923	0.6619	Prednisone	0.2844	Prednisolone
Glibenclamide	NIR2924	0.4768	Tolbutamide	0.1792	Paracetamols
Sorbitol	NIR2925	0.4862	Glycerol	0.1998	Lubritab
Cetylstearyl alcohol	NIR2926	0.7353	Lubritab	0.8187	Lubritab
Fludrocortisone acetate	NIR2927	0.8381	Cortisone acetate	0.4733	Cortisone acetate
Benzoic acid	NIR2928	0.3077	Paracetamol	0.1938	Cortisone acetate
Progesterone	NIR2929	0.6818	Cortisone acetate	0.1543	Precirol
Triamcinolone acetonide	NIR2930	0.5493	Prednisone	0.2192	Prednisone
Salicylic acid	NIR2931	0.1812	Paracetamol	0.1536	Lubritab
Polysorbate 80	NIR2932	0.8776	Macrogol 300	0.7122	Macrogol 300
Propylene glycol	NIR2933	0.6726	Macrogol 300	0.2482	Precirol
Glycerol	NIR2934	0.6465	Macrogol 300	0.8442	Glycerol
Ethyl glycol	NIR2935	0.8475	Macrogol 300	0.4116	Macrogol 300
Cetiol	NIR2936	0.8041	Precirol	0.4686	Precirol
Paraffin liquid	NIR2937	0.8101	Precirol	0.5029	Precirol
Macrogol 400	NIR2938	0.9934	Macrogol 300	0.9651	Macrogol 300
Betamethasone	NIR2939	0.7207	Cortisone acetate	0.3511	Cortisone acetate
Methylprednisolone	NIR2940	0.5296	Prednisone	0.214	Tolbutamide
Gliclazide	NIR2941	0.4351	Prednisone	0.3059	Prednisone
Prednisolone acetate	NIR2942	0.7501	Prednisone	0.2451	Prednisone
Cortisone	NIR2943	0.8026	Cortisone acetate	0.4269	Prednisone
Deoxycortone	NIR2944	0.7375	Cortisone acetate	0.1585	Lubritab
4-Aminosalicylic acid	NIR2946	0.0709	Paracetamol	0.1246	Cortisone acetate
Phenacetin	NIR2948	0.5605	Paracetamol	0.2653	Lubritab
Tolazamide	NIR3070	0.5116	Tolbutamide	0.4014	Tolbutamide
Bumetanide	NIR3071	0.3115	Furosemide	0.1999	Cortisone acetate

3 Conclusions method development and validation

The substances Precirol, Lubritab, prednisone, prednisolone, cortisone acetate, furosemide, tolbutamide, glycerol 85%, and Macrogol 300 could be classified unequivocally by NIRS with WC.

The additional use of another method, e.g. SIMCA, was necessary for differentiation of the different forms of paracetamol.

After internal validation, without conflicts, four methods (A, B, C, and D) were defined for the identification of the 12 substances that are included in the application.

All independent spectra were correctly identified with external validation I. No α or β errors were observed.

With external validation II, conform methods A, B, C, and D, two of the 45 selected substances were incorrectly not rejected (glycerol monostearate and Macrogol 400).

The observed differences among the substances were larger when second-derivative spectra with the selected spectral range of 7000 cm^{-1} – 4000 cm^{-1}were used than when they were not.

In view of the conflict of glycerol monostearate with Precirol in method C, we concluded that the identities of Precirol and Lubritab should be verified by method A. Method C applied with a tighter threshold of 0.98 for these two substances, would also be possible, however in view of the relatively large range in the correlation coefficients of Precirol and macrogol 300 this would probably result in many incorrect rejections, which limits the convenience of the method.

In view of the conflict of macrogol 400 with methods B and C, it was concluded that it should be defined in the description of the application that the NIRS application can not differentiate between the two different homologues 300 and 400 of macrogol. An additional test is required for this, e.g. on viscosity, density, or refraction.

Macrogols concern mixtures of polymers and different forms are defined with different average relative molecular masses. The current monograph of the European Pharmacopoeia – *Macrogols*[23] , which is a general monograph for all forms of macrogol, also includes a test for viscosity in the identification section to differentiate between the different forms of macrogol.

Appendix 4. Formation of the validation set

Substance considered for the validation	Included in set	Justification for absence from the set	Substance included in the NIRS method (object)
4-Aminopyridine	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
4-Aminosalicylic acid micr	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Acetylsalicylic acid < 180 µm	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Aminophenazone	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Beclometasone	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Beclometasone dipropionate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Benorilate	No	Not available: presence at site unlikely	Paracetamol 45 µm, 180 µm, and crystalline
Benzoic acid	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Betametasone dipropionate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Betametasone	Yes	-	Prednisone, prednisolone, and cortisone acetate
Betametasone valerate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Bumetanide	Yes	-	Furosemide
Carbasalate calcium	No	Acid already included	Paracetamol 45 µm, 180 µm, and crystalline
Cetiol V	Yes	-	Glycerol 85%
Cetylstearyl alcohol	Yes	-	Precirol and Lubritab
Chlorbutanole	No	Powder; very different spectrum expected	Glycerol 85%
Clobetasol dipropionate	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Clobetasone butyrate micr	Yes	-	Prednisone, prednisolone, and cortisone acetate
Cortisone	Yes	-	Prednisone, prednisolone, and cortisone acetate
Deoxycortone	Yes	-	Prednisone, prednisolone, and cortisone acetate
Dexametasone disodium phosphate	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Dexametasone micr	Yes	-	Prednisone, prednisolone, and cortisone acetate

Substance considered for the validation	Included in set	Justification for absence from the set	Substance included in the NIRS method (object)
Dimethicone	No	Structure; very different spectrum expected	Glycerol 85%
DMSO	No	Structure; very different spectrum expected	Glycerol 85%
Etacrynic acid	No	Not available	Furosemide
Ethinyl estradiol	Yes	-	Prednisone, prednisolone, and cortisone acetate
Ethyl glycol	Yes	-	Glycerol 85%
Fludrocortisone acetate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Flumetasone pivalate micr	Yes	-	Prednisone, prednisolone, and cortisone acetate
Fluocinolone acetonide	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Fucidic acid	No	Structure; very different spectrum expected	Furosemide
Furadantine	No	Typical colour: Structure; very different spectrum expected	Furosemide
Furazosine (prazosine)	No	Structure; very different spectrum expected	Furosemide
Fusafungine	No	Not available: Structure (polypeptide); very different spectrum expected	Furosemide
Glibenclamide	Yes	-	Tolbutamide
Gliclazide	Yes	-	Tolbutamide
Glipizide	No	Not available	Tolbutamide
Glycopyrrolate	No	Powder; very different spectrum expected	Glycerol 85%
Glycerol	Yes	-	Glycerol 85%
Glycerol diacetate	No	Not available: not likely to be present	Glycerol 85%
Glycerol dichlorhydrine	No	Not available: not likely to be present	Glycerol 85%
Glycerol dimethylketal	No	Not available	Glycerol 85%
Glycerol formal	No	Not available	Glycerol 85%
Glycerol monostearate 46-54	Yes	-	Precirol and Lubritab
Glycerol triacetate	No	Not available	Glycerol 85%
Glyceryl aminobenzoate	No	Semisolid; very different spectrum expected	Glycerol 85%
Glyceryl guaicolate	No	Powder; very different spectrum expected	Glycerol 85%
Glyceryl monoacetate	No	Not available: not likely to be present	Glycerol 85%
Glyceryl tolyl ether	No	Powder; very different spectrum expected	Glycerol 85%
Glycine	No	Powder; very different spectrum expected	Glycerol 85%
Glycol salicylate	No	Not available	Glycerol 85%
Hydrocortisone micr	Yes	-	Prednisone, prednisolone, and cortisone acetate

Substance considered for the validation	Included in set	Justification for absence from the set	Substance included in the NIRS method (object)
Hydrocortisone acetate micr	Yes	-	Prednisone, prednisolone, and cortisone acetate
Ibuprofen	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Macrogol 400	Yes	-	Macrogol 300
Metformine HCl	No	Not available	Tolbutamide
Methyl prednisolone	Yes	-	Prednisone, prednisolone, and cortisone acetate
O-toluamide	No	Explosive; not available	Tolbutamide
Papaverine HCl	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Para-aminobenzoic acid	No	Not available	Paracetamol 45 µm, 180 µm, and crystalline
Paracetaldehyde	No	Liquid; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Paracetamol 500 – 90	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Parachloramine (meclozine HCl)	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Parachlorphenol	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Paracortol	Yes	Is prednisolone	Paracetamol 45 µm, 180 µm, and crystalline
Paradichlorbenzene	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Paraffin	Yes	-	Glycerol 85%
Paraformaldehyde	No	Structure; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Paramethadione	No	Liquid; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Paramethasone	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Paraoxon	No	Liquid; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Parathion	No	Liquid; very different spectrum expected	Paracetamol 45 µm, 180 µm, and crystalline
Phenacetine	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Phenazone	No	Structure; very different spectrum expected: not available	Paracetamol 45 µm, 180 µm, and crystalline
Polysorbate 80	Yes	-	Glycerol 85%
Prednisolone acetate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Prednisolone hemisuccinate	Yes	-	Prednisone, prednisolone, and cortisone acetate

Substance considered for the validation	Included in set	Justification for absence from the set	Substance included in the NIRS method (object)
Prednisolone metasulphobenzoas	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Prednisolone sodium phosphate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Progesterone	Yes	-	Prednisone, prednisolone, and cortisone acetate
Propyfenazone	No	Structure; very different spectrum expected: not available	Paracetamol 45 µm, 180 µm, and crystalline
Propylene glycol	Yes	-	Glycerol 85%
Salicylic acid < 90	Yes	-	Paracetamol 45 µm, 180 µm, and crystalline
Sorbitol apyrogene	Yes	-	Glycerol 85%
Stearic acid pulverised	Yes	-	Precirol and Lubritab
Testosterone propionate	Yes	-	Prednisone, prednisolone, and cortisone acetate
Testosterone micr	No	Number of steroids already included	Prednisone, prednisolone, and cortisone acetate
Tolazamide	Yes	-	Tolbutamide
Tolazoline	No	Structure; very different spectrum expected: not available	Tolbutamide
Tolazoline HCl	No	Structure; very different spectrum expected: not available	Tolbutamide
Tolbutamide sodium	No	Not available	Tolbutamide
Tolcyclamide	No	Not available	Tolbutamide
Tolfenamic acid	No	Structure; very different spectrum expected	Tolbutamide
Tolmetine	No	Not available	Tolbutamide

Appendix 5. Mailing list

1. Directeur-Generaal RIVM
2. Voorzitter van de Gezondheidsraad, Postbus 1236, 2280 CE Rijswijk
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