Near infrared spectroscopy: High speed non-invasive qualification of lyophilized vials

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Introduction

Pharmaceuticals are produced according to the current pharmacopoeias which require the given or stipulated quality parameters to be respected. Usually these are set to certain ranges depending on manufacturing process conditions and are tested using a selected set of samples in quality control.

The typical analysis procedure for solid dosage forms is to bring a small random sample of a production batch to a laboratory, prepare (e.g. grind or dissolve) it and proceed with some form of chemical or spectrometric testing. Not only are the tests destructive, the time required is considerable. If the purpose of the testing is to perform quantitative analysis, then in all probability the batch will have been completed before the results become available to production staff. If samples are rare and qualitative testing is to be performed, the loss of the material is an added drawback to the requisite analysis time.[1]

Moreover, in production it is not just the values of these few samples that are of interest, but whether or not the homogeneity of the batch as a whole within the allowed tolerances. Consequently, quantitative specifications may also be considered from a broader qualitative point of view. Whenever the objective is to test if a certain acceptance limit is respected or not, and this is mainly the case in practice, a classification result is largely sufficient and forms the basis for a 'reject' or 'accept' decision. Chemometrics even allows to approach quantitative problems by a qualitative definition.

The aim of this investigation is to show the feasibility of this approach by using near-infrared spectroscopy (NIRS). Lyophilization of pharmaceuticals for parenteral use is generally performed in a final container such as a glass ampoule or vial. A low residual moisture content in the dried product is essential to maintain the stability of compounds that are prone to hydrolysis.[2, 3] In this case a quantitative residual moisture determination in a lyophilized bottled product is predicted by a qualitative calibration which can subsequently be used for the screening of entire batches to get an insight into the product quality in a broader and more representative way and to improve product safety.[4]

Procedure

The traditional method of release analysis for the determination of water content is volumetric Karl Fischer (KF) titration.[5] As the KF method is time consuming and destructive, it is not suitable for the screening of large numbers of samples. The goal of the current work was to develop an alternative technique that is fast and nondestructive. To this end, a near-infrared (NIR) calibration was developed to provide a rapid and precise technique for the classification of batches into quality groups. Accordingly, the quantitative determination of water content was considered from a qualitative point of view.

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NIRS and principal component analysis (PCA)[6] were applied to classify lyophilized protein vials into two groups of 'good' and 'bad' samples. The acceptance limit for the NIR calibration was set to < 2.0 % to define the group of 'good' samples. Samples with water contents between 2 and 4 % were considered to belong to the group of 'bad' samples which had to be rejected. Any higher values were characterized as 'outliers' and were also rejected.

The method was validated using an extensive set of samples covering a broad moisture range. A qualitative two class model was easy to create and to handle and provided the basis for a 100 % batch control as an alternative to the Karl Fischer titration of selected samples.

Materials and methods

Near-Infrared Spectroscopy

The term NIR refers to the region between the absorption of light energy due to transfer of electrons in the VIS and absorptions due to molecular vibrations in the MIR.[7] The main features observed in the NIR range (780 – 2,500 nm or 12,800 - 4,000 cm^{-1}) are overtones and combinations of the vibrations of CH, OH and NH bonds. [8, 9] All the absorption bands are the result of overtones or combinations of overtones originating in the fundamental mid-range infrared region (2,500 to 50,000 nm or 4,000 to 200 cm^{-1}).[1]

Diffuse reflectance was used in this case; this is currently the most usual measurement technique for NIR-spectroscopy. The incident NIR-light entered the bottom of the sealed vial and interacted with the lyophilized cake. Some radiation was absorbed and some was scattered back. Both effects, absorbance and scattering, provided information about the sample. The spectra obtained contain superposed chemical and physical information about the sample.

An Acousto Optical Tunable Filter (AOTF) spectrometer, Luminar Freespace 3030, with an InGaAs-Detector (Brimrose Corp., Baltimore, USA), was used to carry out the NIR measurements. The size of the measured area was 3 x 5 mm.

Sample Preparation

As the variation in water content of the vials originally produced did not cover the range of water content required to perform a calibration, selected samples were either dried under vacuum or moisturized under an atmosphere of saturated KH_2PO_4.[10]

Conventional analysis

To determine the actual water content in the samples, volumetric Karl-Fischer titration was carried out with a Dosimat 701 KF Titrino (Metrohm AG, Herisau, Switzerland) and Hydranal solvents and titrators by Riedel-de Haën (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany).

Control and Calibration

The Unscrambler® (ver. 7.8, Camo ASA, Oslo, Norway) was applied for the evaluation. In a second step the 'Predict' option of the Snap! software version 2.03 (Brimrose Corp., Baltimore, USA) was utilized for fast and continuous analysis in routine within just one second.

Data acquisition

The sealed glass vials were placed directly on a support 42 mm from the sensor window in order to measure through the bottom of the colorless glass vials. The diffuse reflectance spectra were collected in absorbance mode with a resolution of 2 nm and 10 scans from 1,200 to 2,150 nm being

collected against a 99 % PTFE reference standard without any data pretreatment. To compensate for surface phenomena, the majority of the samples were measured 3 times. The samples were rotated between each measurement. All the measurements were performed at room temperature.

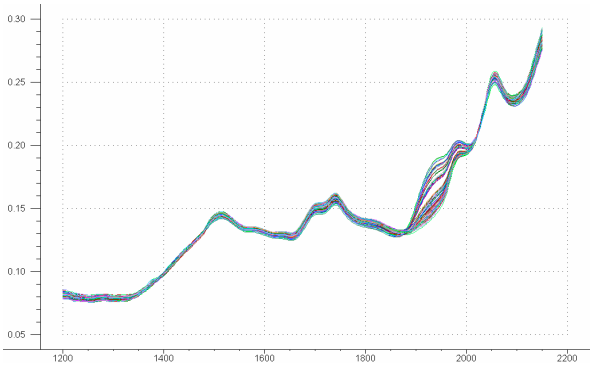

Figure 1: NIR spectra of 'good' and 'bad' samples. The difference at the position of the water band (1,950 nm) is clear. The lower group of spectra corresponds to samples with less than 1.4 % water, the upper group to samples with more than 2.0 %.

Sample selection and calibration

To establish the alternative qualitative calibration, two classification groups of samples were required. In a first step, samples were prepared from different batches with an extended range of water contents. Afterwards the corresponding NIR-spectra were divided into two groups: spectra of 'good' samples containing less than 2 % and spectra of 'bad' samples with more than 2 and less than 4 % water. The 'good' database comprised 69 samples with water contents between 0.3 and 1.4 %. The 'bad' database contained 43 spectra corresponding to water values between 2.0 and 3.5 %. In total a set of only 113 samples was required for modeling.

The gap between these groups was intended to avoid decision uncertainties caused by overlapping analysis results. The size of this interval was defined with respect to the standard error of prediction (SEP = 0.1 %) which would be required for an equivalent quantitative NIR-method. The SEP here was a statistical parameter that indicated the upper limit of accuracy of the NIR-method computed according to equation (1).[6]

$$SEP = \sqrt{\frac{\sum_{i=1}^{N} \left(\hat{y}_{i_i} - y_i\right)^2}{N - 1}} \tag{1}$$

It was considered reasonable to use three times the SEP of the quantitative prediction method to have 99 % confidence. Assuming a normal distribution of the samples included in the calculation, 99 % of them would have an actual water content of y_i (2).

$$y_i \leq \pm 3 \cdot SEP \tag{2}$$

For the qualitative prediction model the difference between the two groups was set to twice this value. Accordingly only calibration samples with water contents of 0.6 % below the acceptance

limit were acknowledged, as it was very important to avoid any false good decisions resulting in a lack of quality.

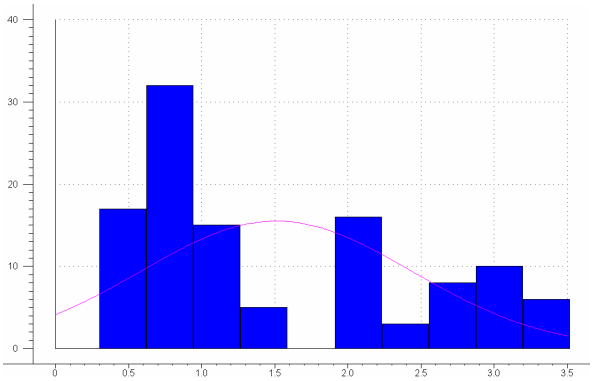

Figure 2: This histogram of the sample distribution illustrates the two distinct calibration sets.

Calibration

In a first step, the feasibility of the calibration was explored by using a third party chemometric software. To carry out the calculation, all the spectra were assembled in one Unscrambler file and a multiplicative scatter correction (MSC) was applied to filter out sampling variations. A PCA was then computed to analyze the correlation between the spectra and the water values. The results showed that differentiation of the qualitative groups of 'good' and 'bad' samples was possible with one single principal component (PC).

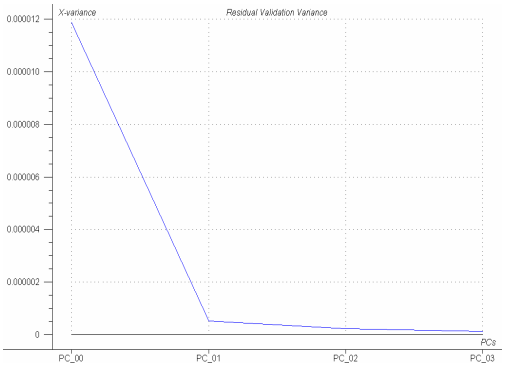

Figure 3: The residual validation variance plot shows that only one PC is required for this calibration.

The second step was the transfer of the calibration to the online software Snap!. The 'Prediction Profile Compiler' was used to create the online prediction operation interface. The two group files were imported to form the basis for the MSC and the qualitative classification. The Mahalanobis distance was fixed to 1.5 to comply with a collection of samples of known water content. To run the prediction, this calibration file was loaded in the 'Predict' module of the Snap! software.

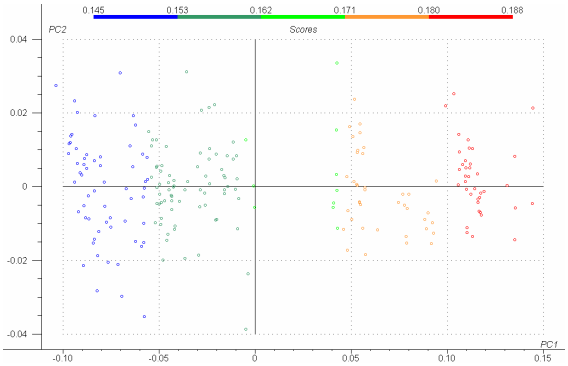

Figure 4: The scores plot confirms the validation variance plot and shows the two separate groups as distinguished by the first principal component.

External Validation

Further samples from different batches of the lyophilized product for validation. The objective was to challenge the calibration with marginal samples. They were first measured with the 'Predict' online monitor, classified on the basis of the NIR-analysis result and then titrated by the KF method.

A total of 150 samples were measured; 75 'good' samples had less than 2 % water (max. 1.7 %), 45 'bad' vials more than 2 % (2.1 - 4.1 %), and 30 'outliers' more than 4 % (4.3 - 10.1 %). All the results were within the specified ranges. The corresponding Karl Fischer water values confirmed the qualitative NIR-calibration.

Results and Discussion

A noninvasive NIR-reflectance method to carry out a good / bad quality assessment with respect to the water content of lyophilized vials has been developed and validated. After mathematical pretreatment, a classification method with an acceptance limit set to 2 % was computed. The calibration set composed of 113 samples was challenged by classifying 150 more samples which were not included into the initial calibration set.

The GMP rules relating to pharmaceutical quality control set high standards for quantitative methods. After general system suitability testing, calibration-specific parameters need to be verified.[11, 12] In particular, the validation e.g. in terms of accuracy, linearity and repeatability with narrow statistic confidence intervals requires considerable work. In NIR assays, the accuracy is indicated by the standard error of prediction. Substantial validation sets over the entire calibration range are used to prove accuracy in the short term and parallel test sets are used to validate the assay over time at regular intervals. Linearity is proven by a straight calibration line over the working range of the assay with correlation coefficients of more than 99 %, slopes close to unity and offsets theoretically equal to zero. These parameters should be submitted to regulatory authorities for registration. Repeatability is demonstrated using several determinations of one or more samples, intermediate precision by conducting assays e.g. on different days and with different analysts.[13] Especially in case of low contents, even minimal deviations lead to high relative standard errors and unacceptable calibrations.

However, only the specificity and robustness need to be validated for qualitative modeling. Accuracy, linearity and repeatability do not need to be evaluated, simplifying the validation effort. On the other hand, potential challenges (e.g. different batches, blends) should be presented to the reference library, but not used to create it, must be tested and shown to give current results. Thus a

new test should be established. Here the threshold situation in particular should be challenged, using samples in the range just above and below the acceptance limit (Table 1), to demonstrate correct assignment by the system.

Traditionally the predicted water value of a quantitative method is compared with an acceptance limit, but the summary of results in the batch record only needs to indicate 'conform' if the sample meets the requirements or 'non-conform' if it does not. However, current regulations require the storage of all the raw data – including the water values. The qualitative method also provides the basis for a release decision but does nor generate this kind of data. An additional advantage would then be the smaller amount of resulting data for storage in the case of large sample quantities possibly reducing the demand of statement by the regulatory authorities.

Table 1. Assessment of validation work relating to quantitative or qualitative calibrations in practice.

Validation	Quantitative	Qualitative
Specificity	+	+
Robustness	+	+
Accuracy	+	--
Precision	+	--
Linearity	+	--
Threshold challenge	**--**	++

Online application

The feasibility of a total online inspection in a lyophilization production line running with a maximum velocity of almost 300 samples per minute was evaluated in a further step. A process simulator was designed to perform all the calibration and validation work offline in a laboratory environment without disturbing the routine procedures. The simulator was a custom-built prototype designed to imitate the real production conditions as closely as possible. The apparatus comprised a stepless controllable servo motor, a starwheel and a rotary encoder similar to an auto sampler. It was possible to select different velocities between 0 and 300 vials per minute. When the measuring position was reached the rotary encoder generated a trigger signal that started the measurement by the spectrometer. The trigger signal was a function of the starwheel position and was therefore time independent. Consequently the measurement always started on the same spot on the bottom of the vials, irrespective of the rotation speed.

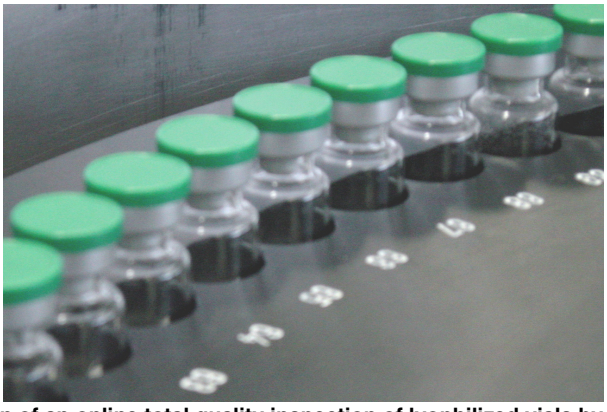

Figure 5: Simulation of an online total quality inspection of lyophilized vials by NIRS.

The continuously moving samples were scanned five times through the bottoms of the vials. This produced an average spectrum made up of scans distributed over the product surface to compensate for potential in-homogeneity. When the new samples were predicted by the online software both the spectra and the prediction results were saved simultaneously. Once the calibration work was complete and a GMP compliant validation wad completed the entire system was ready to be integrated in the production line.

In future, new calibrations for new products or changed compositions requiring re-calibration can be performed on a second equivalent spectrometer in the laboratory environment. These models need to be transferred to the plant spectrometer afterwards, assuming good agreement between of the two devices in terms of spectral quality. As calibration transfer from one spectrometer to the other within one type series including method modification and revalidation still remains an issue, simple and robust calibration techniques are of interest.[14] If the required calibration work is limited the product could be remodeled independently on a second or third device.

Conclusions

A qualitative two-class model is easy to create and to handle compared with quantitative determinations. No large-scale data treatment is necessary, the sample sets can be kept very small, but the calibration remains fast and robust. This NIRA method was nondestructive and fast. Each analysis took less than one second.

For these reasons, the NIR-application could be used not just for laboratory quality control, but also for the screening of large numbers of samples or even for a total quality control of entire batches. Online measurements with variable velocities up to 300 vials per minute were feasible. Samples deviating from the quality parameters could be rejected simultaneously and a homogeneous in-specification batch would result. Only this kind of testing would really give the desired information about the global quality of the complete lot. With NIR spectroscopy, the pharmaceutical industry will move one step closer to "zero-defect" quality control, making the costs associated with the method's development well spent.[15]

Thus the use of a qualitative calibration in quality control or process monitoring offers an opportunity to be grasped.

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