

## Application Bulletin 358/1 e

# Analysis of residual moisture in a lyophilized pharmaceutical product by near-infrared spectroscopy

### Branch

Pharmaceutical

### Keywords

Near-infrared spectroscopy, lyophilized pharmaceuticals, water determination, Karl Fischer titration, loss on drying (LOD)

### Summary

This Application Bulletin describes an approach for developing near-infrared methods for the determination of residual moisture in typical lyophilized pharmaceutical products. In this case, samples were contained in sealed 30 mL serum vials (3 cm o.d.). The average cake weight of this product was 1.6 g with a target moisture level of 1% and acceptable moisture limit of not more than 2% by weight. A calibration sample set was prepared by spiking sample vials with varying amounts of water. Diffuse reflectance near-infrared spectra of these calibration samples were acquired and multiple linear regression (MLR) algorithms applied to correlate spectral changes to experimentally measured moisture values as determined by Karl Fischer titration.

### Introduction

Quantification of residual moisture in lyophilized parenteral products is an important measurement in the pharmaceutical industry. Analyses are routinely performed for process control and to insure that samples from production lots meet specified release parameters. For developmental purposes, such measurements are necessary during stability studies and optimization of the freeze/pump cycles used in the freeze-drying process.

Traditional techniques for measuring moisture include Karl Fischer titration, gas chromatography, and thermogravimetric analysis, also known as loss-on-drying (LOD). Although these methods offer certain advantages with respect to sensitivity, solvent specificity, and system cost, they all suffer from the same drawbacks of being time consuming and destructive to the sample being analyzed.

Near-infrared (NIR) spectroscopy has been shown to be an ideal method for rapidly measuring moisture in freeze-dried materials. Typical analysis times are less than 30 s. Calibrations for quantifying trace amounts of moisture are possible, due to the strong overtone absorption bands for

water at 1940 nm and 1450 nm. In a diffuse reflectance configuration, the measurement may be performed through the bottom of the vial, since the borosilicate glass that is commonly used in ampoules and serum vials is «invisible» in the near-infrared region. Therefore, little or no sample preparation is required, and the need for costly or toxic reagents is eliminated. The technique is nondestructive and noninvasive; attributes that are particularly advantageous when analyzing samples containing expensive active ingredients, or samples that may be used for stability studies since the same vials can be used for subsequent potency assays.



**Figure 1:** Rapid Content Analyzer (RCA) sampling module used to analyze lyophilized material

### Experimental

Near-infrared spectra were acquired using a FOSS NIRSystems Model 6500 spectrometer equipped with a Rapid Content™ Analyzer (RCA), Figure 1. The RCA sampling accessory utilizes a mechanical iris to reproducibly position vials over the monochromatic incident beam and an array of lead-sulfide detectors capable of analyzing diffusely reflected near-infrared radiation from 1100 nm to 2500 nm. Spectral data were signal averaged using 32 scans. Since this instrument is not available anymore, the NIRS XDS RapidContent Analyzer is recommended. Data collection and analysis was performed using the Vision™ software.

A calibration set was prepared using 46 sample vials, which were divided into ten groups, A to J. Samples in each group were spiked with specified amounts of water to provide incremental increases in the moisture content as shown in Table 1.

**Table 1:** Calibration Sample Set

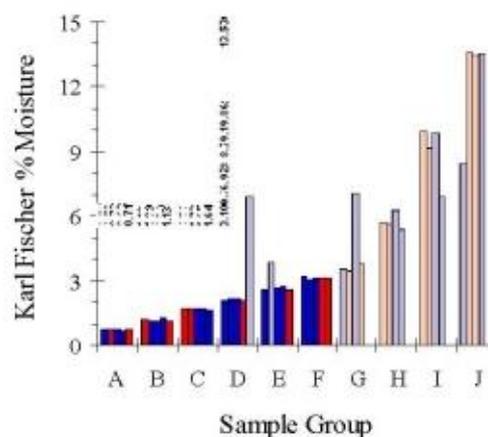
Group	No. of Vials	Desired H <sub>2</sub> O Increase [% by weight]	Volume of H <sub>2</sub> O to add [μL]
A	5	0.0	0
B	5	0.5	8
C	5	1.0	16
D	5	1.5	24
E	5	2.0	32
F	5	2.4	40
G	4	2.9	48
H	4	4.8	80
I	4	9.1	160
J	4	13.0	240


**Figure 2:** Schematic of the technique used to spike sample vials with water.

The procedure used to spike the samples is illustrated in Figure 2, and involved holding the sample vials horizontally at a slight angle while injecting a measured volume of distilled water into the headspace via a microsyringe. The thin needle of the microsyringe was accommodated by using a larger bore needle (18 gauge) as a sheath, which was inserted through the rubber stopper. The samples were then stored for 48 hours to allow the moisture to fully evaporate and equilibrate with the sample in the vial. During this time, the tilted orientation of the vials was maintained so that the water did not come into direct contact with the freeze-dried cake. This procedure readily allows for the preparation of calibration sets which have a «box-car» distribution, with roughly the same number of samples in each «group» of moisture levels. This approach also proved to be more convenient than procedures which utilize humidity chambers, since prior knowledge of the moisture uptake rate is not necessary.

The moisture content of calibration samples used for developing the NIR calibration equations were determined by volumetric Karl Fischer titration. No sample preparation was required.

## Results and discussion


**Figure 3.** Karl Fischer analysis results of calibration set samples. The blue and red bars indicate «training» and «test» set samples, respectively.

### Karl Fischer titration of calibration samples

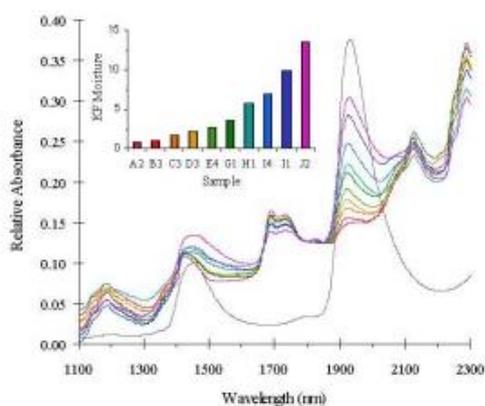
The results from the Karl Fischer analyses are shown in the bar chart of Figure 3. The measured values are consistent with the desired increase for each group, indicating that the experimental approach used to spike the samples can be easily controlled. However, in three samples, D5, E2, and G3, the measured moisture values were higher than expected. This can be attributed to either the addition of excess water or high initial moisture content in each vial prior to spiking. Excluding these samples, the average moisture content before spiking was determined to be approximately 0.7% by subtracting the desired moisture increase from the actual measured value for each vial.

### Near-Infrared spectral data

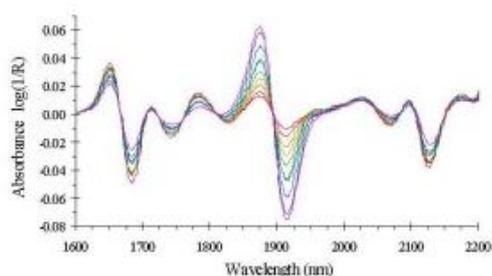
Figure 4 shows the NIR spectra of ten calibration samples containing varying amounts of moisture as indicated in the inset bar graph. For presentation purposes, each spectrum was offset corrected by subtracting the corresponding absorbance at 1820 nm from each datapoint, and the scale was then reset relative to the lowest overall absorbance being zero. The correlation between an increase in moisture content and the change in the spectral features around the band at 1940 nm is evident.

Second derivative spectra are often used for developing quantitative regression equations. This math pretreatment is used to correct for sloping backgrounds and varying offsets associated with differences in the reflectivity of each samples sample. These variations are caused by physical parameters, such as differences in particle size distributions

and cake appearance. Figure 5 shows the second derivative spectra of the same ten samples shown in Figure 4.



**Figure 4:** Diffuse reflectance near-infrared spectra of lyophilized samples containing varying amounts of moisture as indicated in the inset bar graph. Spectra were offset corrected relative to the absorbance values at 1820 nm. The dash line is a transmission spectrum of water, with a pathlength of 0.3 mm and absorbances scaled to 20% the actual values.



**Figure 5:** Second derivative spectra corresponding to the absorption spectra of Figure 4

### Regression equation development

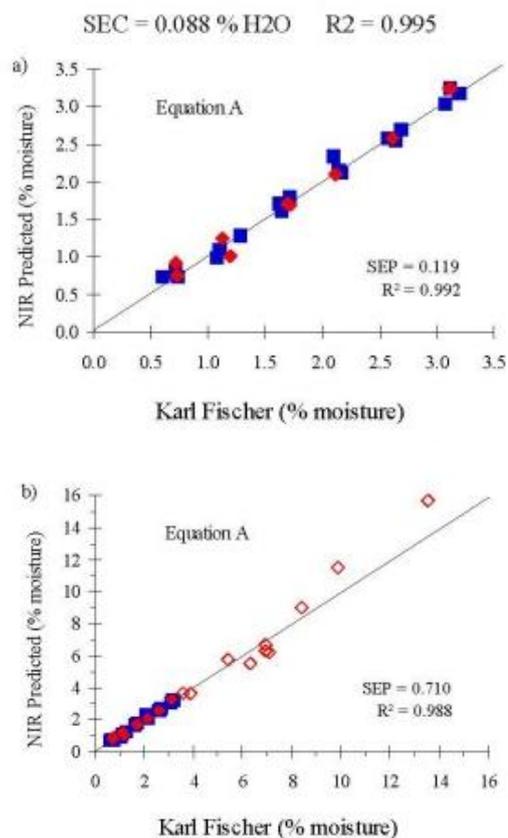
When developing quantitative models, spectra of the calibration samples are typically divided into two distinct groups. Samples in the «training-set» are used to develop the regression equations, while samples in the «test-set» are used to challenge or validate the equations. Spectra of a particular sample should not be used in both sets. For this application, the blue and red bars of the graph in Figure 3 indicate training and test set samples, respectively.

To obtain a calibration for the analysis of samples having moisture values within a range near the specified acceptance limit, i.e., ~ 2% H<sub>2</sub>O, multiple linear regression (MLR) algorithms were applied to second derivative spectra of calibration samples from groups A to F. This yielded calibration Equation A, which is optimized for samples having low moisture content, i.e., 0% to 3.5% H<sub>2</sub>O. The

divisor term minimizes effects due to multiplicative scatter caused by pathlength difference in the samples.

### Equation A: For samples with low residual moisture

$$\text{NIR Predicted moisture in \%} = 0.806 - 19.411 \left( \frac{A''_{1842 \text{ nm}}}{A''_{2124 \text{ nm}}} \right)$$

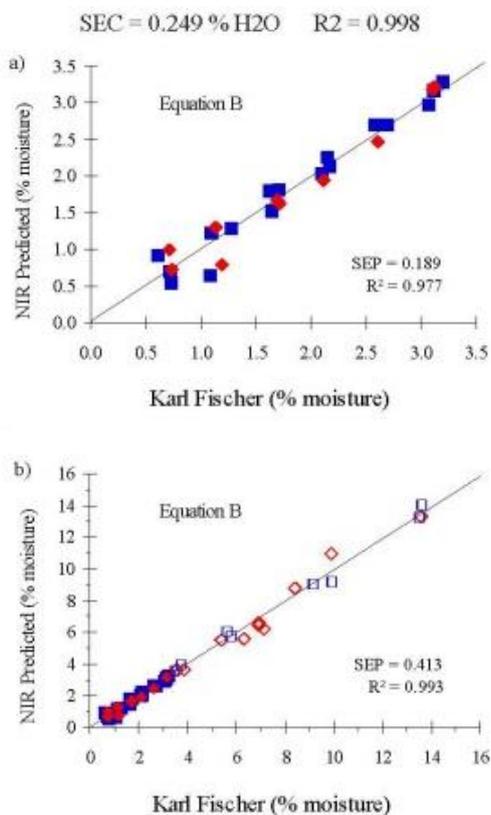


**Figure 6.** NIR predicted moisture values determined using Equation A vs. Karl Fischer results. Training-set samples are indicated by the blue squares, test-set samples are indicated by the red diamonds. The top graph shows the results at lower moisture values, over which the equation was optimized.

A second calibration was developed using MLR and second derivative spectra of all of the samples in the training set. This provided Equation B, which is optimized for samples containing high moisture, with levels up to 15% H<sub>2</sub>O.

### Equation B: For samples with high residual moisture

$$\text{NIR predicted Moisture in \%} = 1.427 + 6.472 \left( \frac{A''_{1842 \text{ nm}}}{A''_{2162 \text{ nm}}} \right)$$

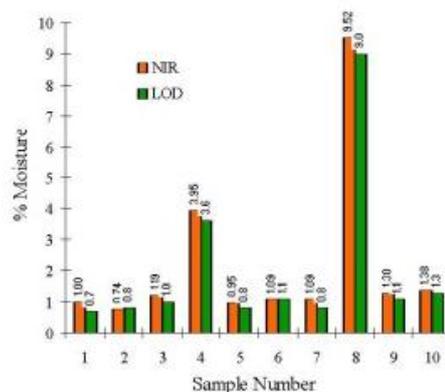


**Figure 7:** NIR predicted moisture values determined using Equation B vs. Karl Fischer results. Training-set samples are indicated by the blue squares, test-set samples are indicated by the red diamonds. The bottom graph shows the results at higher moisture values, over which the equation was optimized. At the lower moisture values the prediction did not perform as well as Equation A, see Figure 6.

Both regression equations yielded excellent coefficients of determination, with  $R^2 > 0.99$ . The standard error of calibration (SEC) for equation B was slightly larger than equation A, due to the extended range over which the calibration was developed. As expected, equation A performed quite well in predicting the moisture content of samples in the test set having values less than 3.5% H<sub>2</sub>O, and yielded a relatively low standard error of prediction, SEP = 0.119% H<sub>2</sub>O. However, when applied to test set samples containing higher moisture levels, > 8% H<sub>2</sub>O, equation A tended to overestimate the actual amount of moisture, as was measured by Karl Fischer titration. In such cases it would be appropriate to apply equation B, which proved to be more accurate for samples with high moisture levels, but was less precise when applied to samples containing relative low residual moisture levels.

### Comparison of NIR to LOD

Loss-on-drying (LOD) is a method that is commonly used to measure residual solvents in powders and lyophilized materials. An experiment was performed to compare the results of the NIR method described above with those from LOD analysis. This involved analyzing ten samples using the two methods. Two of the ten samples were known to have relatively high moisture levels. The results are shown in the bar graph of Figure 8.



**Figure 8:** Comparison of NIR and LOD analyses for ten additional lyophilized samples. Two of the vials were known to have high moisture levels. For these samples, equation B was applied to obtain the NIR result.

There is a good correlation between the NIR and LOD results. However, on average, the NIR values were slightly higher than the corresponding LOD results by  $0.20 \pm 0.17\%$ . This bias is present because Karl Fischer titration was used as the «primary method» when developing the NIR regression equations. Loss-on-drying employs a gravimetric approach to measure an equilibrium process. Because of hydrogen bonding interactions, it is virtually impossible to remove all of the surface water from a substance by simply drying it in an oven for an extended period of time. Subsequently, LOD will typically yield lower residual moisture values than Karl Fischer titration.

The correlation between the NIR and LOD methods can be improved by applying a simple bias correction to the equation. This would be appropriate, since the differences are explainable, and appear to be consistent over the moisture ranges for which the equations were developed.

### NIR Analysis of Defect Samples Containing «Melts»

A common defect that may be observed in lyophilized products is «meltback» or a collapse of the freeze-dried cake caused by partial dissolution. This will occur if the lyophilization process is incomplete and there are high residual moisture levels in the vials. Melts can also develop during the freeze-drying process if the temperature and/or

pressure are not maintained below the transition point where liquefaction occurs. The resulting cakes will usually appear very different from an ideal cake, which should have uniform color and texture. Samples containing meltback will typically be smaller with nonuniform appearances. However, if the freeze-dried cake is relatively large, it is possible that the melt may be contained entirely within the interior of the cake, since that is the region where the last bit of moisture is removed from the sample. In such cases, it may be difficult to detect the defect simply by visual inspection. A sample containing a melt is considered to be a «critical defect», since degradation of the active drug will likely occur, thus affecting the efficacy of the final product.

The NIR method was used to analyze a group of fifteen production samples that had been identified as having melts. In each case, the samples were found to have high moisture, with levels ranging from 5 to 20% H<sub>2</sub>O. The NIR method was able to identify these samples as having high moisture, despite the fact that the sample matrix was significantly compromised, i.e., diffusely reflected near-infrared radiation from a sample containing a melt may be very different from one that does not. Although the actual moisture levels of these samples were not determined, the results are significant, since they demonstrate that the NIR method can also be used as a qualitative inspection tool. Since the analysis is nondestructive, it would be feasible to automate the analyses, such that every production sample is examined.

### Conclusions and recommendations

Near-infrared spectroscopy is ideally suited for the analysis of residual moisture in lyophilized products. Calibration samples can be easily generated by injecting specific amounts water directly into the headspace of the vials and allowing the moisture to equilibrate with the freeze-dried cakes. This procedure provides a convenient means of preparing calibration sets with an even distribution of moisture levels. Regression equations can be optimized in order to provide measurements of residual moisture within different ranges, i.e., high and low % H<sub>2</sub>O values, if the training sets are comprised of samples with appropriate moisture levels. Biases may be observed when comparing the NIR predictions with the results of methods that were not used to generate the regression equations. In many cases, these differences can be explained, and subsequently corrected for by applying a bias adjustment to the calibration equation. Because the NIR method is nondestructive, it is possible to reanalyze samples by using a second method. This is particularly useful when analyzing samples used for

stability studies, or to verify high moisture content in samples that are found to have moisture contents above the specified amount. The speed and ease of the technique allows for the analysis of very large sample sets. By automating the analysis, it would be feasible to incorporate residual moisture testing as an integral part of the quality assurance testing of all lyophilized vials.