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Applying spectral peak area analysis in near-infrared spectroscopy moisture assays

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Abstract

Spectral peak area analysis has in this study been shown to be a viable method in near-infrared spectroscopy (NIRS) moisture assays. The study also shows that the required number of calibration samples can be minimized, and the method is, therefore, especially suitable for moisture assays in early formulation development and in-situ process monitoring.

Diffuse NIRS was utilized in the development of moisture assays for the model compounds polyvinylpyrrolidone and hydroxypropyl-β-cyclodextrin and also for a lyophilized formulation. Reference data were obtained using coulometric Karl Fischer titration. The NIRS measurements were performed through the bottoms of the sample vials using either a Fourier Transform-Near-Infrared (FT-NIR) spectrometer fitted with a diffuse reflectance probe or a dispersive single beam spectrometer. The ratios of the peak areas of a water peak at 5200 cm^{-1} and a reference peak were evaluated using linear regression analysis. The spectral peak area analysis method was compared with a conventional partial least squares regression method. The moisture assays were verified using independent test sets. The investigated moisture range was 0–22% for the samples of PVP, 0–8.5% for the samples of hydroxypropyl-β-cyclodextrin and 0.5–8.5% for the samples of the lyophilized formulation.

The results of the spectral peak area analysis and the conventional partial least squares regression were similar, but the peak area method was more robust and could also make accurate predictions for lyophilized PVP samples, although the calibration set consisted of non-lyophilized samples. The peak area method required fewer calibration samples than the conventional partial least squares regression method.

Keywords: Near-infrared spectroscopy; Moisture; Spectral peak area analysis; Partial least-squares regression; Lyophilization

1. Introduction

Moisture assays of pharmaceutical formulations were among the earliest applications of near-infrared spectroscopy (NIRS). NIRS is well suited for measurement of moisture because water has strong absorption bands in the near-infrared spectrum. A moisture assay of a compound or a formulation by near-infrared diffuse reflectance spectroscopy has been demonstrated to be rapid, non-invasive, non-destructive and accurate and has, therefore, been widely applied as a quality control method for manufactured compounds or formulations [1–8]. NIRS has in recent years also been used for in-line process monitoring of moisture content [9–11].

Moisture content can be very important for the chemical and/or physical stability of compounds and formulations, especially for lyophilized formulations. The active substance is often an article in short supply in early development, but it is at the same time very important to characterize the compound and formulations that are being developed. A non-invasive and non-destructive tool such as NIRS is, therefore, very valuable in this development phase, because additional analytical tests can be performed with the same samples. It would be desirable to be able to use an accurate prediction model with a minimum set of calibration samples to facilitate determinations of moisture content as early as possible in the development work.

The magnitude of noise and variability is greater in in-line process monitoring situations compared with off-line quality control measurements because the measuring situation is not as optimal. More extensive sets of calibration samples are, therefore, required in order to incorporate the greater spectral

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variability. Prediction models with a minimal need for calibration samples would, thus, be especially beneficial for process monitoring situations. Another obstacle with in-line monitoring is that it can be difficult to generate in-process calibrations samples with different moisture contents. Robust calibration models that can use samples generated off-line and, therefore, different from in-process samples would, thus, be beneficial.

Multivariate data analysis is generally applied for near-infrared spectra because highly overlapping absorption bands, descending from weak overtone and combination bands, hamper the assignment of a signal to certain functional groups. A disadvantage with multivariate analysis is however that the prediction model often requires an extensive calibration set because all possible sample variability should be incorporated into the calibration set to achieve optimal performance. Substantial time and effort is, therefore, often required in the development of the prediction model, and it is important that the calibration samples are representative of the real analytical samples.

A moisture assay based on direct spectral peak analysis has the potential to require a minimal calibration set. There are examples where the absorbance at one or multiple specific wavelengths [1] have been used in such assays. Accuracy and robustness of such univariate models are, however, most likely less good than multivariate data analysis models.

To the best of our knowledge, there are so far no reports on NIRS moisture assays based on spectral peak area analysis. The objective of this work was, therefore, to evaluate and demonstrate the feasibility of such a procedure.

2. Materials and methods

2.1. Materials

The compounds and the lyophilized formulation were packed in 5 or 10 ml injection vials made of uncoloured borosilicate glass tubing of Ph. Eur. and USP type 1 (Münnerstädter Glaswarenfabrik, Germany). The vials were closed with 14 or 20 mm bromobutyl rubber stoppers designed for lyophilization applications (Helvoet Pharma Belgium NV). The model compounds used were polyvinylpyrrolidone (PVP) i.e. Polyvidon K25 (BASF, Germany) and hydroxypropyl-β-cyclodextrin, i.e. Kleptose (Roquette). A lyophilized formulation (AstraZeneca R&D Mölndal) was used as a model formulation.

2.2. Instrumentation

NIR diffuse reflectance spectra were acquired with either a Fourier Transform-Near-Infrared (FT-NIR) spectrometer (Bomem NetworkIR) or a dispersive single beam spectrometer (NIRSystems Model 6500 spectrometer). The FT-NIR spectrometer was fitted with a 500-μm single fiber diffuse reflectance probe and a thermo-electrically-cooled InGaAs detector. The dispersive instrument was equipped with a Rapid Content Analyzer (RCA).

Karl Fischer (KF) titration was performed with a 737 KF Coulometer (Metrohm, Switzerland) or the same coulometer equipped with an 832 Thermoprep device (Metrohm, Switzerland). In the coulometric titration, HYDRANAL-Coulomat AG (anolyte) and HYDRANAL-Coulomat CG (catholyte) (Riedel-de Haën, Germany) were used in the titration cell.

2.3. Samples

Samples of 50–150 mg of PVP were weighed into 10 ml glass vials. The samples were dried in a vacuum oven at 50 °C and 5 mbar during 3 days. The vials were sealed with rubber stoppers that had been dried together with the samples in the vacuum oven. Some of the dried samples were analyzed directly after the drying process. Other samples were exposed to humid air during different time intervals in order to acquire different levels of moisture content. The relative humidity of the air was either uncontrolled, i.e. ambient, or controlled by a saturated solution in a sealed container. The solutes used in the saturated solutions were phosphopentaoxide, lithium chloride, magnesium chloride, potassium chloride and magnesium nitrate. The saturated solutions resulted in a relative air humidity of approximately 5, 17, 38, 48 and 57%, respectively. A set of 62 samples was analyzed, and half of this set, uniformly spanning the moisture range studied, was used as a calibration set, and the other half was used as a test set.

Samples of 40–280 mg of lyophilized PVP were manufactured. Aliquots of 4 ml of 4–7% w/w solution of PVP were filled into 10 ml glass vials, which were semi-stoppered. The samples were lyophilized and stoppered in the lyophilizer. The samples were either dried to different moisture content during the lyophilization process or exposed to ambient air during different time intervals in order to acquire different levels of moisture content. A set of 14 lyophilized PVP samples was analyzed and used as an additional test set to check if the tested calibration methods would be able to predict the moisture content correctly even though the samples were structurally different than those of the calibration set.

Samples of 60–90 mg of hydroxypropyl-β-cyclodextrin were weighed into 5 ml glass vials and sealed with rubber stoppers. The samples were exposed to ambient air during different time intervals in order to acquire different levels of moisture content. A few of the samples that had been analyzed in the Thermoprep device and thereby dried at 135 °C were reanalyzed directly. A set of 55 samples of hydroxypropyl-β-cyclodextrin was analyzed in total, and half of this set (i.e. 27 samples), uniformly spanning the moisture range studied, was used as a calibration set, and the other half (i.e. 28 samples) was used as a test set.

Samples of approximately 50 mg of a lyophilized formulation were manufactured. Aliquots of 1 ml of approximately 4% w/w solution of the drug substance were filled into 5 ml glass vials, which were semi-stoppered. The samples were lyophilized and stoppered in the lyophilizer. The samples were either lyophilized to different moisture content or exposed to ambient air during different time intervals in order to acquire different levels of moisture content. A set of 262 samples of the lyophilized formulation was analyzed in total and approximately one fourth of this set, i.e. 65 samples, uniformly spanning the moisture range studied, was used as a calibration set, and the rest was used as a test set.

2.4. Coulometric Karl Fischer titration

Two different methods of Karl Fischer titration were used in this study. In one of the methods, the whole content in the vial was dissolved in approximately 5 ml of anolyte, which was injected through the stopper of the vial with a disposable syringe. The anolyte solution was then transferred back to the titration vessel of the Coulometer. The contents in the vial were determined by weighing the vial before and after the Karl Fischer titration. The vials were rinsed and dried before being reweighed after the titration. All of the lyophilized samples were analyzed according to this method.

In the other method, the moisture content was determined using a Thermoprep device connected to the Coulometer. The sample was heated to 135 °C in the oven block and the moisture thereby released in the form of water vapor, which was transferred to the titration vessel by a dry carrier gas. The carrier gas stream was produced by a built-in air pump.

2.5. Spectrum collection and data analysis

All spectra were recorded through the bottoms of the sample vials prior to KF titration. Duplicate measurements were made with the FT-NIR spectrometer, and the sample vial was rotated into a different position between the measurements. Each spectrum from the FT-NIR spectrometer was the average of 20 scans over the wavenumber range 4000–9000 cm^{-1} (1111–2500 nm) with a resolution of 16 cm^{-1}. One single spectrum was recorded of each sample with the dispersive single beam spectrometer. Each spectrum from the dispersive single beam spectrometer was the average of 32 scans over the wavelength range 1100–2500 nm (4003–9091 cm^{-1}) with a bandwidth of 10 nm and a data point interval of 2 nm. Spectral data have been recalculated and reported in wavenumbers, i.e. in the unit cm^{-1}.

All of the samples of PVP and hydroxypropyl-β-cyclodextrin were analyzed with the FT-NIR spectrometer, and all of the samples of the lyophilized formulation were analyzed with the dispersive single beam spectrometer. A set of 9 hydroxypropyl-β-cyclodextrin samples was also analyzed with the dispersive spectrometer and used as an additional test set to check if the calibration method would be able to predict the moisture content correctly even though the samples were analyzed with a different spectrometer.

The software package Grams/32 (Galactic Industries, USA), accompanying the FT-NIR instrument, was used to acquire the spectra from that instrument. The software package Vision, accompanying the dispersive single beam spectrometer, was used to acquire the spectra from that instrument. Partial least squares regression (PLSR), including second derivative spectral pre-treatment, was performed with The Unscrambler version 7.6 (Camo Inc., Norway). The second derivative was calculated using the Norris method with 4-9 data point averaging. The calculation of standard normal variate (SNV), transformation of the spectral data, and calculation of peak areas, peak heights and linear regression of peak area ratios or peak height ratios were performed using Matlab version 7.0 (The Mathworks, Inc., USA).

The results obtained in the moisture prediction were expressed as the relative standard error of prediction (RSEP):

$$\mathrm{RSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\mathrm{H_2O\_KF}_i - \mathrm{H_2O\_NIR}_i)^2}{\sum_{i=1}^{n}\mathrm{H_2O\_KF}_i^2}}$$

Where n is the number of samples, and $\mathrm{H_2O\_KF}_i$ and $\mathrm{H_2O\_NIR}_i$ are the water contents as determined by KF titration and the NIR method, respectively. RSEPC and RSEPT are used to refer to the relative standard error of prediction for the samples used in the calibration set and the test set, respectively.

3. Results and discussion

3.1. Moisture assay by NIRS using spectral peak area analysis

3.1.1. Peak area determination

Overlap of spectral bands is typical in the NIR region. Water, however, displays specific bands at 5200 cm^{-1} (1940 nm, OH combination band) and 6900 cm^{-1} (1440 nm, band of the first overtone of the OH stretch), which have been very useful in the study of the state of water in various samples. These absorption bands, especially the water band at 5200 cm^{-1}, are not only strong but also typically well resolved from absorption bands of other compounds. The exact position and width of the water peak resulting from the OH combination bands vary slightly, depending on the chemical and physical environment. It can be seen in Fig. 1 that the water peak varies depending on the kind of solid matrix and the moisture level. It is clear by visual inspection of these spectra that a quantitative relationship exists between the level of absorption and the moisture content. It can also be seen that there is one spectral peak around 5700–5800 cm^{-1} that is constant at the different water levels. This peak, therefore, correlates to the chemical compounds and not to water. The shape of the reference peak is different for each kind of sample investigated.

The described spectral characteristics were used in an intuitive manner in the spectral peak area analysis described here. That is, it was assumed that the ratio between the area of the water peak and the area of the reference peak correlates with the water content. It was also assumed that such an approach would improve the robustness of the method.

Each spectrum was normalized in the spectral range used, applying SNV transformation. The correction factors for the SNV transformation, i.e. the mean and the standard deviation, were determined solely in the reference peak region, which means that the reference peak was used as the normalization standard. The area under the curve was determined for the water peak and the reference peak, respectively. The peak area was determined by using cubic spline data interpolation of the spectral data in the region of each peak. The local maximum of the peak and local minima, representing the start and the end of the peak, respectively, were determined as illustrated in Fig. 2. The start and the end of the peak were slightly adjusted so that the base line of the peak became a tangent to the curve at both

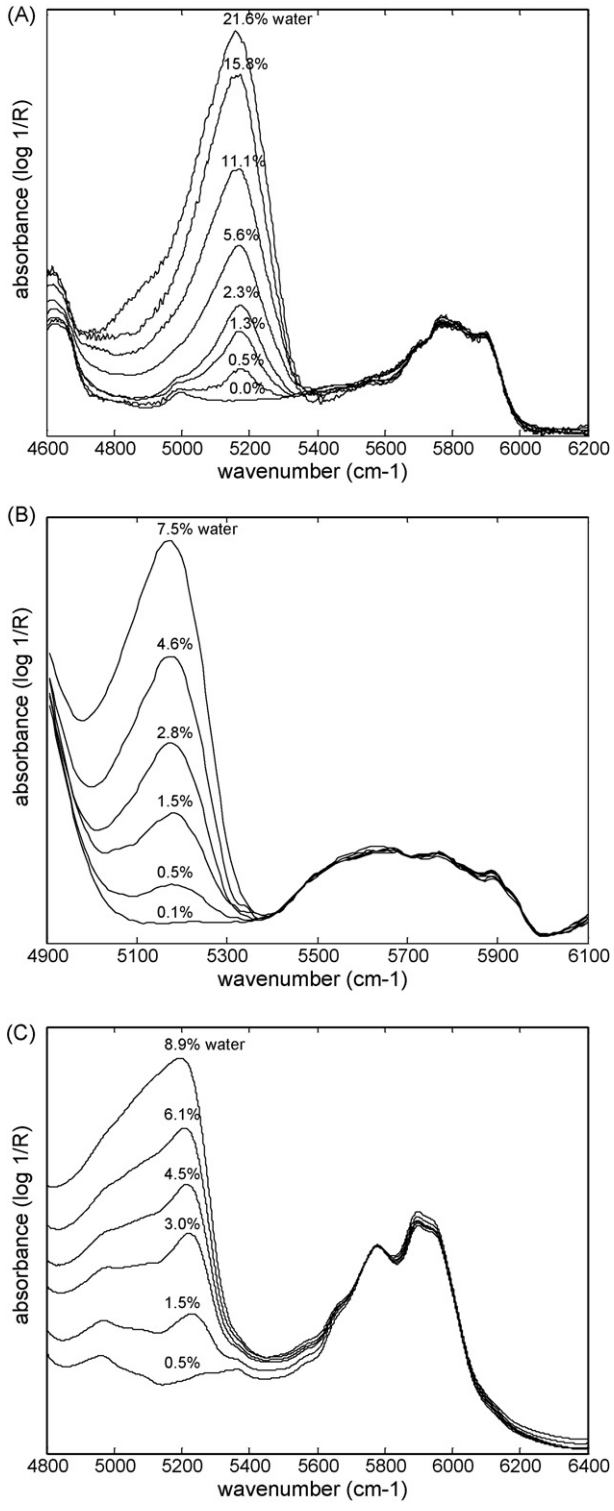

Fig. 1. SNV pretreated spectra of samples of (A) PVP; (B) hydroxypropyl-β-cyclodextrin; and (C) the lyophilized formulation with different moisture levels (expressed as percent w/w) in the spectral range of the water peak at 5200 cm^{-1} and the reference peak around 5700–5800 cm^{-1}.

locations as shown in Fig. 2. The area under the curve between the start and the end of the peak was determined by numerical integration of the interpolated data using a recursive adaptive Simpson quadrature standard routine. The area below the baseline of the peak, i.e. the triangle below the baseline between the start and the end of the peak, was subtracted from the peak area.

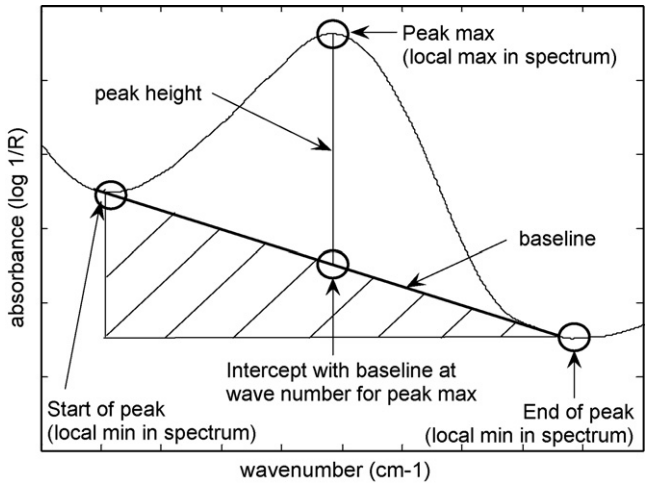

Fig. 2. Illustration of peak area determination.

3.1.2. Calibration functions

Fig. 3 shows the peak area ratio for the calibration samples as a function of the moisture determined by KF titration. It is necessary to use two different linear calibration functions for the samples of PVP, one for low moisture content and another for high moisture content, in order to fit the data well. Only one linear calibration function was necessary for the samples of hydroxypropyl-β-cyclodextrin and the lyophilized formulation. Fig. 4 shows second derivative spectra of each kind of sample with different moisture content. The water peak is bimodal with increasing moisture content for PVP but singular for both hydroxypropyl-β-cyclodextrin and the lyophilized formulation. The reason is most likely that water is differently associated with PVP at higher moisture content than at low moisture content, i.e. bound water at low moisture content and surface water at higher moisture content. The spectral peak area analysis can, thus, also give qualitative information about the moisture in the samples.

3.1.3. Test data sets

Moisture correlation plots of all of the calibration data sets and the test data sets are presented in Fig. 5. The regression analysis indicates a good correlation between predicted and measured moisture, i.e. the slopes of the correlation lines are close to one and the *y*-intercepts are close to zero. The RSEPC and RSEPT values are similar, which shows that the correlations are similar for the test sets compared with the calibration sets. The RSEP values are lowest for the lyophilized formulation, i.e. approximately 5%. The RSEP values for PVP and hydroxypropyl-β-cyclodextrin are significantly higher, i.e. between 8 and 9%.

3.1.4. Additional test data sets

Two additional test data sets, 9 samples of hydroxypropyl-β-cyclodextrin analyzed with the alternative NIR instrument,

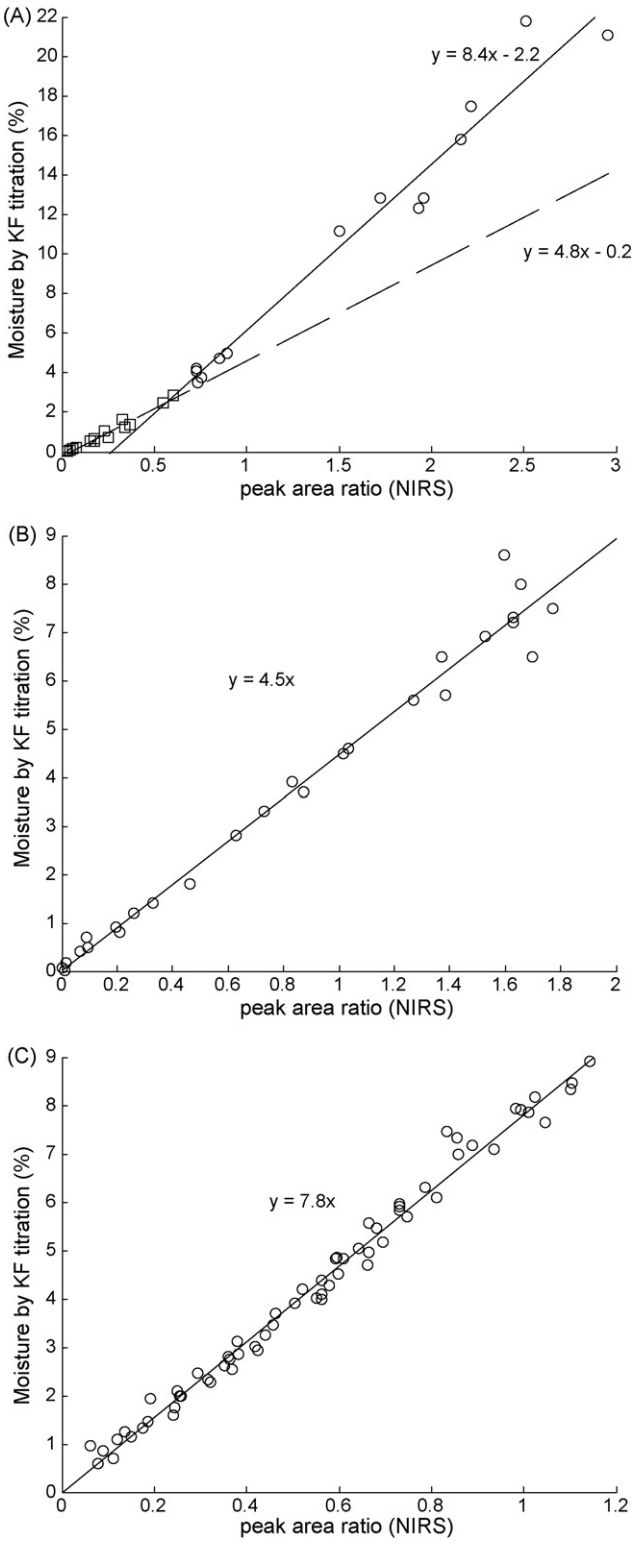

Fig. 3. Plots of the correlation of the peak area ratio and the determined moisture by KF titration for the calibration set for (A) PVP; (B) hydroxypropyl-β-cyclodextrin; and (C) the lyophilized formulation. Data in the low moisture range for the samples of PVP are indicated in figure (A) by the square marker symbol.

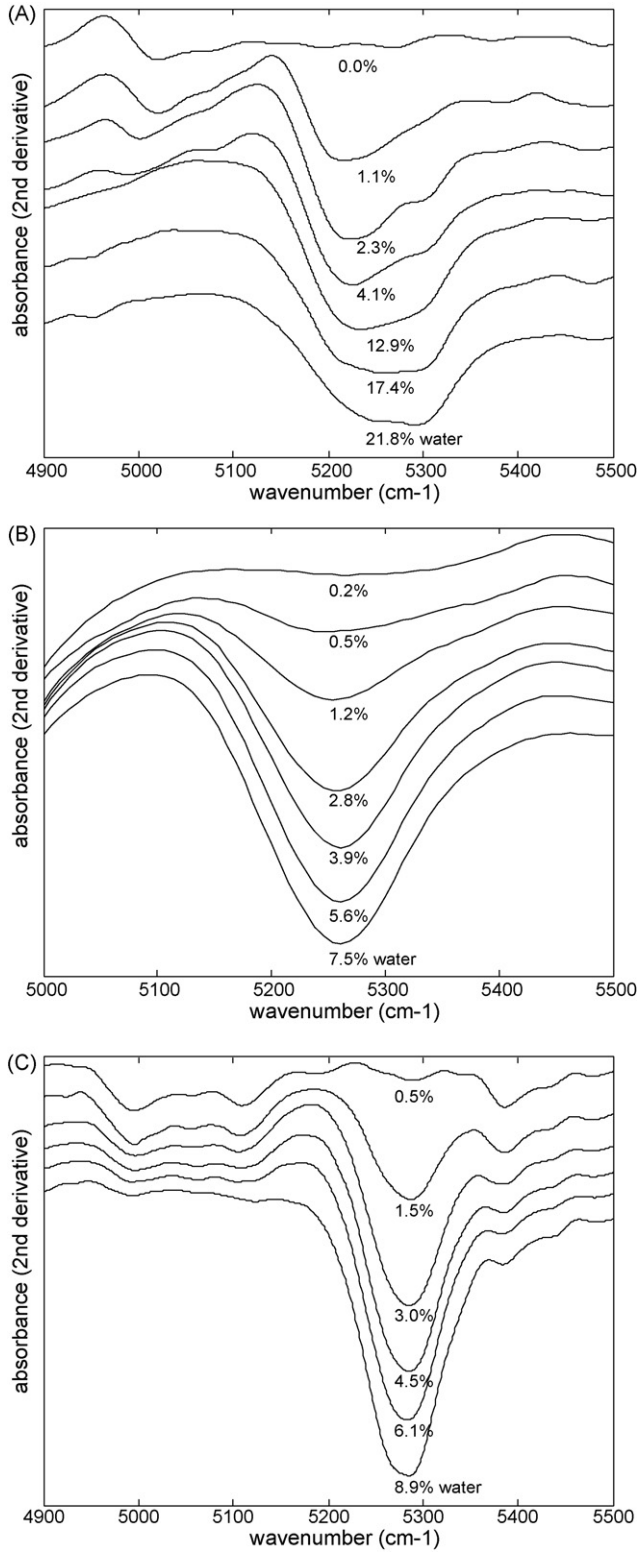

Fig. 4. Second derivative spectra of samples of (A) PVP; (B) hydroxypropyl-β-cyclodextrin; and (C) the lyophilized formulation with different moisture levels (expressed as percent w/w) in the spectral range of the specific water band at 5200 cm^{-1}.

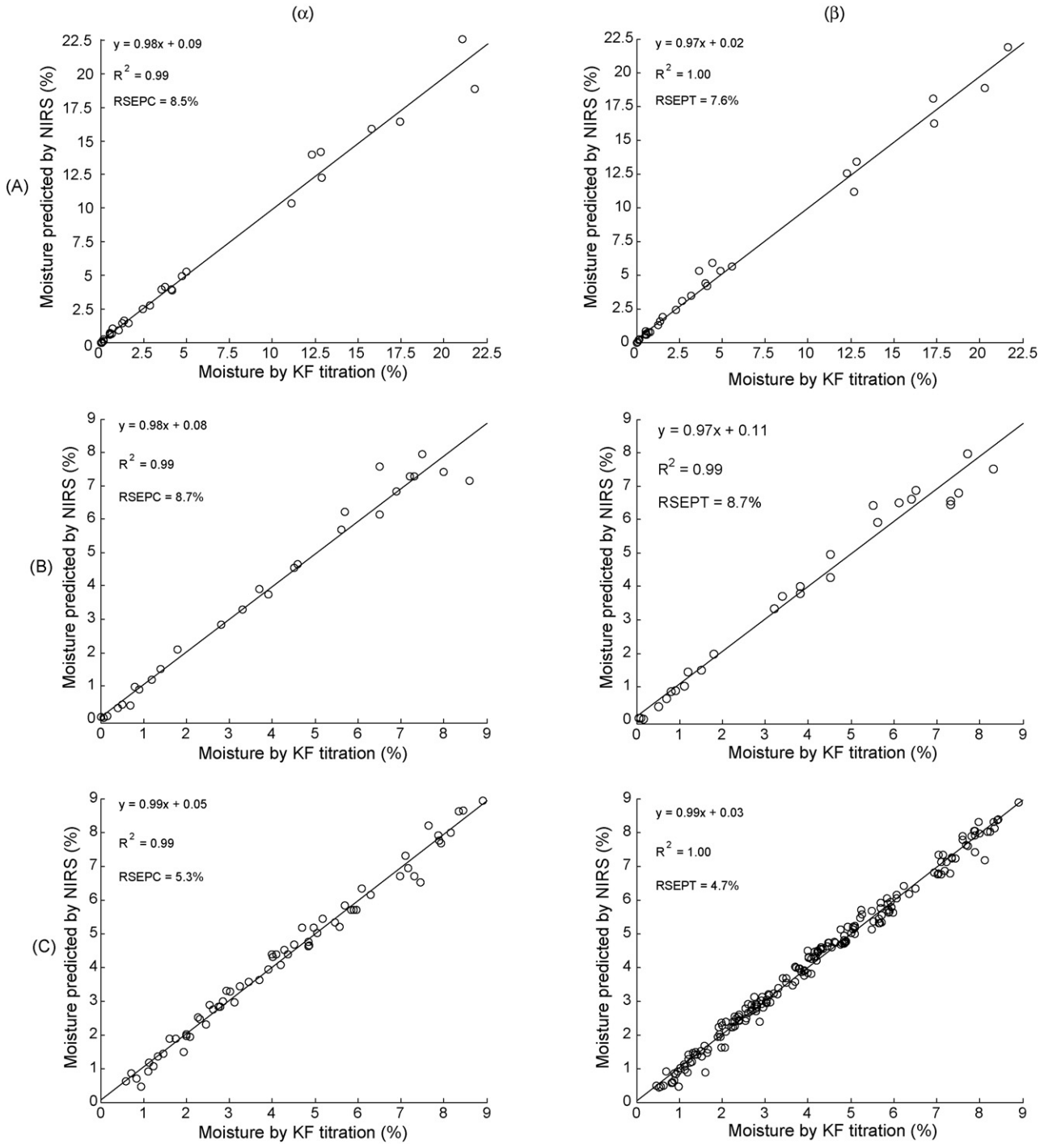

Fig. 5. Plots of the correlation of the moisture in samples of (A) PVP; (B) hydroxypropyl-β-cyclodextrin; and (C) the lyophilized formulation predicted by NIRS using peak area analysis and determined by KF titration for (α) the calibration sets and (β) the test sets.

i.e. the dispersive single beam spectrometer, and 14 samples of lyophilized PVP were assayed using the calibration functions in Fig. 3, and the results are presented in Table 1.

The correlation between predicted and measured moisture is good for both additional sample sets. It should be pointed out that the macroscopic structure of the lyophilized and non-lyophilized PVP samples is different, i.e. the lyophilized material is much more diluted. The shape of the NIRS spectra of the two different categories of PVP samples is the same but the amplitudes of the spectra of the lyophilized samples are smaller compared with the non-lyophilized samples. The moisture assay is robust enough to make correct predictions also for the lyophilized samples, which was not the case for the PLSR method.

The correlation between predicted and measured moisture in the samples of hydroxypropyl-β-cyclodextrin analyzed with the dispersive single beam spectrometer is also good and similar to the results obtained with the FT-NIR spectrometer.

Table 1
Results obtained using spectral peak area analysis or PLSR in the moisture assays of the two additional test data sets

Samples	Peak area RSEPT (%)	PLSR RSEPT (%)	Spectrometer
Lyophilized PVP	7.6	80.9	FT-NIR
Hydroxypropyl-β-cyclodextrin	7.4	9.0	Dispersive single beam[a]

[a] Calibration set analyzed with the alternative spectrometer.

3.1.5. Using an alternative reference peak

There can often be more than one suitable reference peak in the spectrum to choose between, and this is the case for the spectra of hydroxypropyl-β-cyclodextrin and the lyophilized formulation. The chosen reference peak for PVP is, however, the only one obvious in the spectrum for that compound. The alternative reference peak for hydroxypropyl-β-cyclodextrin and the lyophilized formulation were at 4800 cm^{-1} and 4200 cm^{-1}, respectively. Both alternative peaks were at lower wavenumbers than the water peak at 5200 cm^{-1}. These alternative reference peaks were applied in the moisture assays of the calibration data sets and the test data sets. New peak area ratios, calibration functions and moisture contents were determined. The results of the predictions obtained with the alternative reference peaks are presented in Table 2. The results using the alternative reference peaks are similar to the results using the original reference peaks, and this shows that the method is robust concerning the choice of reference peak.

It is probably better to choose the reference peak with the most constant peak area with varying moisture content, and the reference peak should not be too small or too big compared with the water peak because that will most likely make the method more sensitive to noise and measurement variations. It is, thus, informative to look at the water peak area and the reference peak area separately, because that will make it is easier to determine the most appropriate reference peak and to detect outliers in the sample set.

3.1.6. Using peak height instead of peak area

An alternative to using peak area ratio in the spectral analysis would be to use peak height ratio. This alternative was tested, and the peak height was determined in the same calculation loop as for the peak area. The base line calculation was the same, and a determination of the peak maximum and the intercept with the base line at the peak maximum was added. The results of the predictions for all of the calibration data sets and the test data sets are presented and compared with peak area ratio results in Table 3. The correlation between predicted and measured moisture is significantly worse compared with the peak area results. It can, therefore, be concluded that the peak area was a better option than the peak height. The absorbance bands of water are, due to hydrogen bonding, broad and may experience wavelength shifts due to matrix interactions, and this will make univariate methods, such as the peak height method, less suitable than multivariate methods such as the peak area method.

Table 2
Results obtained using alternative reference peaks in the moisture assay for hydroxypropyl-β-cyclodextrin and the lyophilized formulation

Samples	RSEPC (%)	RSEPT (%)
Hydroxypropyl-β-cyclodextrin	6.5	10.3
Lyophilized formulation	4.7	5.2

Table 3
Results obtained using peak height ratio or peak area ratio in the moisture assay of the calibration data sets and the test data sets

Samples	Peak height		Peak area	
	RSEPC (%)	RSEPT (%)	RSEPC (%)	RSEPT (%)
PVP	15.6	11.3	8.5	7.6
Hydroxypropyl-β-cyclodextrin	11.9	12.3	8.7	8.7
Lyophilized formulation	6.4	6.6	5.3	4.7

Table 4
Results obtained when the calibration set for the lyophilized formulation was gradually reduced and the water content of the test set was predicted

No. of calibration samples	Peak area RSEPT (%)	PLSR RSEPT (%)
65	4.7	4.1
33	4.7	4.1
4	4.8	9.4
2	4.7	25.6

3.1.7. Test of the robustness of the calibration model by reducing the calibration set

The robustness of the calibration model for hydroxypropyl-β-cyclodextrin and the lyophilized formulation was tested by removing samples in the calibration sets. The calibration function was recalculated after the reduction, and the water content of the test set was predicted using the new calibration function. The test sets were kept constant, i.e. the same as shown in Fig. 5. The results from this test are shown in Tables 4 and 5. It is obvious that the calibration set can be greatly reduced and that the calibration model, therefore, is very robust. It is, thus, possible to generate a good calibration model using only two calibration samples. Only one calibration sample is actually needed in these two cases because both calibration functions intersect the origin.

Table 5
Results obtained when the calibration set for hydroxypropyl-β-cyclodextrin was gradually reduced and the water content of the test set was predicted

No. of calibration samples	Peak area RSEPT (%)	PLSR RSEPT (%)
28	8.7	9.5
14	8.9	10.1
4	9.7	13.1
2	8.7	22.8

Table 6
Results obtained using PLSR and different numbers of PLS components in the moisture assay of the calibration data sets and the test data sets

Samples	3 components		4 components		5 components	
	RSEPC (%)	RSEPT (%)	RSEPC (%)	RSEPT (%)	RSEPC (%)	RSEPT (%)
PVP	6.2	9.1	4.5	9.2	2.1	8.0
Hydroxypropyl-β-cyclodextrin	8.8	11.6	5.8	9.9	5.1	9.5
Lyophilized formulation	4.1	5.1	3.3	4.1	3.3	4.1

3.1.8. General remarks about the spectral peak area analysis method

A moisture assay using spectral peak area analysis will require a minimum of calibration samples, i.e. just enough samples to determine the linear calibration function or functions. The method can also be used without any calibration samples to make qualitative predictions. It will also be possible to do initial quantitative predictions by assuming that the calibration function will be the same as for another compound or formulation. Such initial assumptions can be checked by a qualitative comparison of the spectral data, and it will be possible to make better initial assumptions when calibration functions for many different compounds and formulations have been obtained. Spectral peak area analysis will also facilitate one or multiple point moisture analysis calibration of the NIR instrument using one or multiple reference samples with known moisture content.

3.2. Moisture assay by NIRS using partial least-squares regression

3.2.1. Calibration and test data sets

Spectral pre-treatment before applying PLSR has been shown to improve the assay. In this work, second derivative, standard normal variate (SNV) transformation and no pre-treatment were tested (data not shown). It was concluded that SNV transformation gave the best result, and it was chosen as the pre-treatment method.

PLSR enables the use of the whole spectral wavelength range of the data set. It may, with a calibration set consisting of only a few samples, be advisable to reduce the number of variables by discarding those that contribute no information on the analyte. In this work, a reduced range (4400–6500 cm^{-1}) and the whole spectrum (4000–9000 cm^{-1}) were tested (data not shown). It was concluded that the whole spectrum gave the best result, and it was decided to use the whole spectrum.

As for the spectral peak area analysis, it was assumed that it would be better to use two different calibration models for the samples of PVP, one for moistures up to 3% and another for moistures above 3%. The concept of using one calibration model for low moistures and another for high moistures was, therefore, used for these samples although this is not practical for PLSR because it is necessary to know beforehand which calibration function to apply. It would, however, be possible to first use a classification methodology to determine qualitatively whether a sample has high or low moisture and thereafter use PLSR to quantify the moisture.

A first estimation of the optimum number of PLS factors was obtained by means of cross validation testing, and the results were 3 factors for PVP and the lyophilized formulation and 4 factors for hydroxypropyl-β-cyclodextrin. Additional PLS factors were, however, tested and the results are shown in Table 6. It was concluded that one additional PLS factor beyond the optimum number obtained by means of cross validation improved the predictions; i.e. it reduced RSEPC and/or RSEPT. The chosen number of PLS factors was, thus, four for PVP and the lyophilized formulation and five for hydroxypropyl-β-cyclodextrin.

The moisture correlation plots of the calibration data sets and the test data sets are presented in Fig. 6. The regression analysis indicates a good correlation between predicted and measured moisture. The quality of the predictions is similar to the results from the spectral peak area analysis.

One difference for PLSR compared with the spectral peak area analysis method is that RSEPC in general is significantly lower than RSEPT. This indicates that there is a risk with PLSR that the prediction error for new samples can be significantly higher than with the calibration set. Another difference is that the PLSR method seems to be less robust considering that a few of the test samples were poorly predicted, as can be seen in Fig. 6.

3.2.2. Additional test data sets

Two additional test data sets, 9 samples of hydroxypropyl-β-cyclodextrin analyzed with the alternative NIR instrument, i.e. the dispersive single beam spectrometer, and 14 samples of lyophilized PVP were assayed, and the results are presented and compared with results from the spectral peak area method in Table 1.

The correlation between predicted and measured moisture in the samples of hydroxypropyl-β-cyclodextrin is similar to what was obtained with the test data set measured with the FT-NIR spectrometer. The predictions are, however, unsatisfactory for the samples of lyophilized PVP. The prediction error is significantly higher for a few of these samples, and the RSEPT value is, therefore, unsatisfactorily high. The lyophilized samples are different than the calibration data set, and the PLSR method is not robust enough to make correct predictions for such deviating samples.

3.2.3. Test of the robustness of the calibration model by reducing the calibration set

The robustness of the calibration model for hydroxypropyl-β-cyclodextrin and the lyophilized formulation was tested by

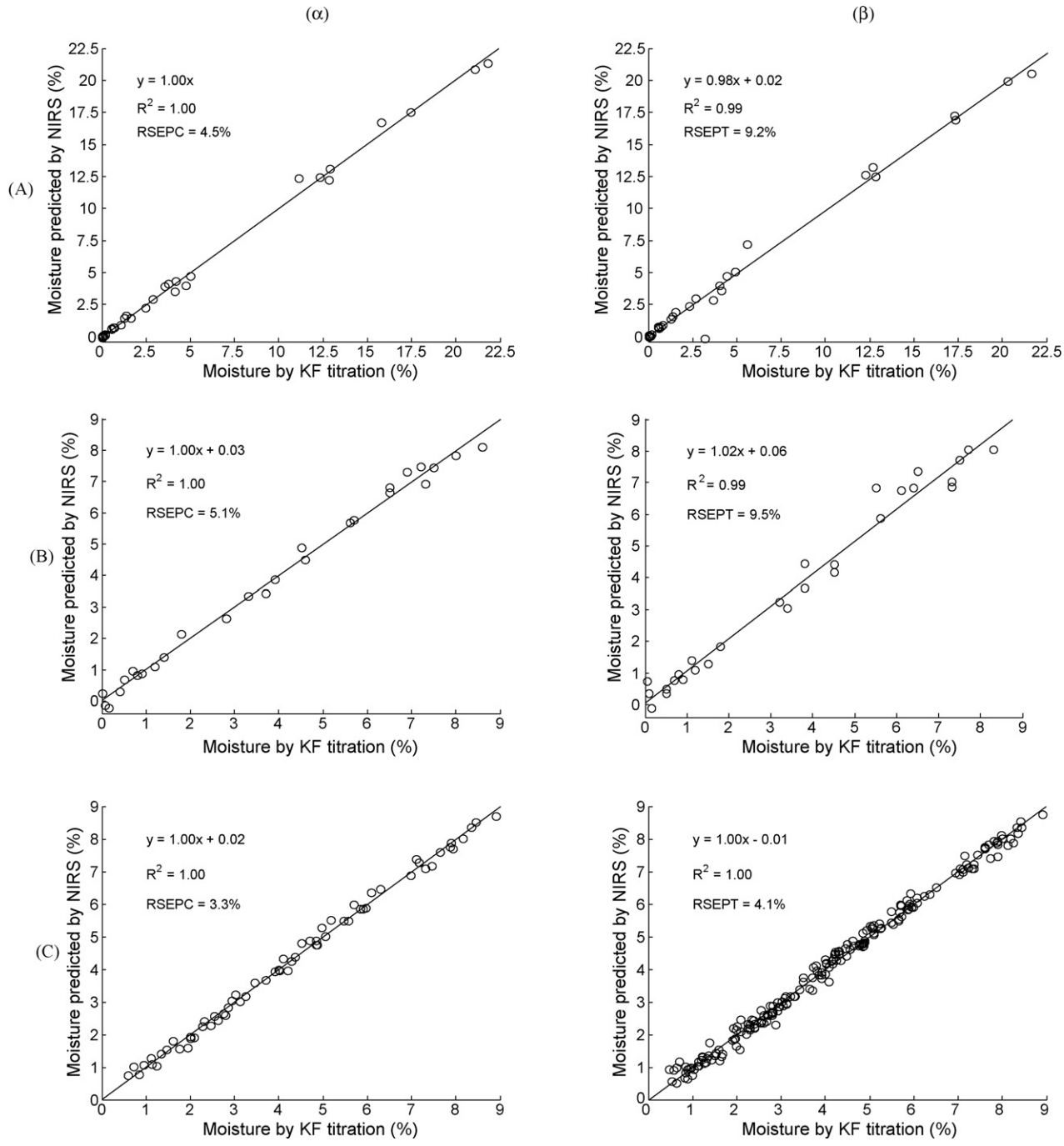

Fig. 6. Plots of the correlation of the moisture in samples of (A) PVP; (B) hydroxypropyl-β-cyclodextrin; and (C) the lyophilized formulation predicted by NIRS using PLSR and determined by KF titration for (α) the calibration sets and (β) the test sets.

removing samples in the calibration sets. The calibration model was recalculated after the reduction, and the water content of the test set was predicted using the new calibration model. The test sets were kept constant, i.e. the same as shown in Fig. 6. The results from this test are presented and compared with results from the spectral peak area method in Tables 4 and 5. It is obvious that the calibration set can be reduced significantly but not to the extent that is possible with the spectral peak area method. It is necessary to have more than just a few samples to generate a reliable PLSR calibration model.

4. Conclusions

Spectral peak area analysis has been shown to be a viable method in near-infrared spectroscopy moisture assays. The spectral characteristics of the NIR spectra were used in an intuitive manner, i.e. it was assumed that the relative size of the water peak compared with the reference peak correlated with the water content. It can, however, be informative to look at the water peak area and the reference peak area separately, because that makes it easier to determine the most

appropriate reference peak and to detect outliers in the sample set.

An alternative to using peak area in the spectral analysis would be to use the peak height, but in this study, peak area proved to be a better option than peak height.

This study showed that spectral peak area analysis gave similar results as partial least squares regression. The spectral peak area analysis method was, however, more robust and could make accurate predictions for lyophilized PVP samples although the calibration set consisted of non-lyophilized PVP samples. The method is less sensitive to sample variations than PLSR. Spectral peak area analysis will facilitate using minimal calibration sets and will be especially suitable for moisture assays used in early formulation development and in-situ process monitoring.

Spectral peak area analysis will also facilitate one or multiple point moisture analysis calibration of the NIR instrument, using one or multiple reference samples with known moisture content.

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