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Development and transferability of near-infrared methods for determination of moisture in a freeze-dried injection product*

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Abstract: Near-infrared reflectance (NIR) methods for non-invasive and non-destructive measurement of moisture in a lyophilized product were developed independently at two sites. NIR spectra were collected with the same model instrument at each site. The spectra were scanned through the bases of unopened glass vials using a horizontal instrument accessory. The primary reference data for moisture content were generated using Karl–Fischer titration on the same individual vials. The NIR calibration equations were developed with second derivative spectral data using regression programs within the NIR software. These calibration equations were validated using independent test data from additional vials of product from the original site, and cross tested against similar data from the other site to check their robustness. This cross comparison demonstrated that a calibration equation from one site could satisfactorily be used for predicting moisture contents in product manufactured at the other site. Expected variations arising from differences in vial material, manufacturing process, analysts and instruments appeared to be satisfactorily accommodated. Finally, two test data sets were obtained by scanning a set of samples on two spectrometers at one site. The results from these data sets were comparable using the calibration equation developed solely on one of the two instruments. The results from all calibration and test sets are presented and discussed, and an assessment of the method transferability between instruments and sites is given.

Keywords: *Near infrared; NIR; spectrometry; reflectance; moisture; freeze-dried product.*

Introduction

Many injectable products are currently prepared in closed vials as freeze-dried solids, the moisture content of which is an important control parameter in the product specification. Karl–Fischer (KF) titrations are commonly used to determine the water content, but are time-consuming and labour-intensive and can be prone to errors arising from interference from atmospheric moisture.

Near-infrared (NIR) spectrometry is well suited to the measurement of water since O—H overtone and combination bands are pronounced in this region of the spectrum. NIR is also readily adaptable to reflectance work, and the low intensities of NIR absorptions consequently permit the direct measurement of water over wide concentration ranges in solid samples. NIR scans can easily be obtained through the walls of glass vessels with minimal interference or spectral scatter.

NIR methods for the determination of water in a freeze-dried injection product were developed by scanning the spectra of the intact freeze-dried solid through the bases of unopened vials. NIR is essentially a secondary technique, as the response must be calibrated against reference analytical data. This reference data must be obtained on a calibration set of samples that incorporates all characteristic variations of real samples that subsequently will be analysed. In the present work, NIR calibrations were set up, using KF titration reference data, on separate instruments at Wellcome laboratories in the USA and the UK. Each of the calibration equations was tested against independent test sample sets before being electronically transferred to the other site for testing against the local indepen-

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dent test sets. In addition, a similar transferability study was carried out between two instruments at the USA site.

Experimental

Apparatus

All three NIR spectrometers used in this work were NIRSystems model 6500, and were equipped with a horizontal set-up module (HSM) and NSAS software. KF titrations were carried out using a Metrohm 701 KF Titrino semi-automated titrator in the USA laboratory and a Metrohm E547 Automat titrator in the UK.

Reagents

Hydranal Composite 5 (Reidel de Haen) KF reagent and HPLC-grade methanol (Fisher or Baxter) were used in KF determinations in the USA laboratory. The same KF reagent was used in the UK, but the methanol used was Specially Dried GPR grade (BDH).

Samples

In all cases, samples were obtained from production batches. In the UK study, 40 vials were available, two from each of 20 different production batches. A sample-select option in the NIR software was used to select particular vials to cover 95% of the variation detected in the NIR spectra, and the 28 vials selected were used to establish the calibration set; the remaining 12 were retained as an independent test set.

In the USA study, more samples were available, and a different approach was tried. Thirty-two vials from four batches of product were taken. In an attempt to increase the moisture content and widen the range of the calibration set, the rubber closures of 11 vials were pierced with an 18-gauge needle and the vials were exposed to high humidity for up to 116 h. USA test samples were set up independently of the calibration set. Three sets were selected: set 1 comprised 25 samples taken from the same four production batches as the calibration set, whereas sets 2 and 3 comprised 27 and 19 samples, respectively, obtained from five other production batches. Details of all the sample sets are shown in Table 1.

Table 1
Water content of calibration and test sets

Sample set	Number in set	Water content (% w/w) by KF		
		Mean	Range	% RSD
Calibration sets				
UK	28	1.66	0.90–3.10	33
USA	32	2.11	1.34–4.90	33
Test sets				
UK	12	1.59	1.16–2.13	20
USA1	25	1.84	1.27–2.70	20
USA2	27	1.35	0.84–1.71	16
USA3	19	1.39	1.12–1.71	12

Product is manufactured to the same formula at the USA and UK sites and filled into glass vials that differ slightly between the sites. There are undoubtedly minor processing differences at each site. Additionally, the samples used in this study were filled to give a 500 mg dose in the USA batches and a 250 mg dose in the UK batches. Thus, there are a number of variations between the products at each site that provided an interesting challenge for the transferability of NIR methods.

Method

All vials were individually marked and scanned on the NIR spectrometer prior to analysis by KF titration. The spectrometer was placed in a horizontal position with the optic window facing upwards and fitted with the HSM. This accessory allowed samples to be enclosed in the dark during scanning. A black anodized aluminium template was used to reproducibly centre the unopened product vials on the spectrometer window, and the NIR scans were taken through the bases of the vials. The instrument parameters used were: replicate sample scans (25 in USA, 32 in UK) automatically averaged to provide sample spectrum; reflectance detector on ×1 setting; white ceramic reference; scan range 1100–2500 nm; and manual sample cell operation. Reference scans were taken before the sample scans in all cases.

Data processing

Each sample was stored as an electronic data file on the computer hard disk and the NSAS software was used to append the KF water content to the file as reference data. Zero-order NIR spectra were transformed to second derivative spectra in order to remove absorbance offsets which resulted from slight particle size variations or compaction in the freeze-dried solid and from optical aberrations due to the glass vials.

Equations were generated from the calibration data sets. The USA calibration equation used a single wavelength based on the well-defined O—H combination band at 1920 nm, whereas the UK equation utilized two wavelengths based on the same O—H band at 1914 nm and a software-selected 1340 nm band, presumably arising from C—H bands from the active ingredient. This second wavelength provided a means of accommodating matrix effects.

These calibration equations were tested using the independent test sets at the originating site before transferring calibration equations electronically between instruments and sites. Each transferred calibration equation was installed on the other instrument, and the calibration data set of the receiving site was used to enable the slope and bias adjustments within the software to be applied to the equation to correct for slight differences between instruments. In the UK, the USA calibration data set was installed on the UK instrument and a dual wavelength calibration equation was forcibly generated. Additionally, a calibration equation transfer was undertaken between two instruments at the USA site. The adjusted equation was then used to predict results on the local test sets.

Precision studies were undertaken in the USA whereby repeatability was assessed from eight replicate NIR determinations on each of six samples on two instruments. Analyst-to-analyst reproducibility was also assessed by two analysts each performing separate determinations on 11 samples with the same instrument.

Results and Discussion

Initial calibrations

The calibration equations generated at the USA and UK sites were as follows:

USA: % moisture = 2.008 − 144.876 (D_{1920}),
UK: % moisture = 2.117 − 118.5 (D_{1914}) − 53.75 (D_{1340}),

where D represents the second derivative deflection at the indicated wavelengths; this is derived from the log $1/R'$ values (essentially absorbances for reflectance spectra) of the original zero-order spectra.

These calibration data are presented as scatter plots in Fig. 1(a) and (b), with the ideal correlation line between NIR and KF ($y = x$) overlaid to illustrate the goodness of fit of the data to an ideal correlation. To validate each calibration equation at the originating site, NIR prediction data were generated for the independent test sets and compared with the KF reference data. An example of typical test set data is shown graphically in Fig. 2(a), again with the ideal correlation line overlaid, and the residual data (difference between NIR and KF values) are summarized for all within-site test sets in Table 2.

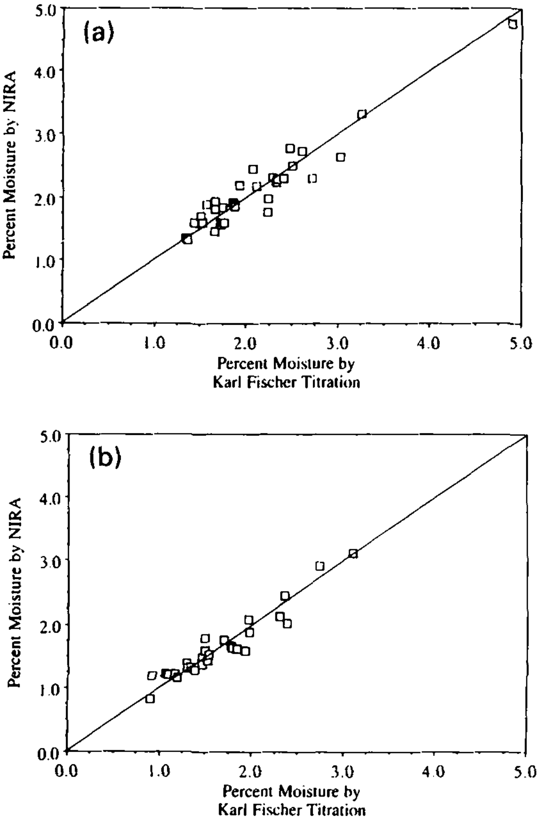

Figure 1
(a) USA single wavelength calibration plot. (b) UK dual wavelength calibration plot. (NIRA = Near-infrared reflectance analysis.)

Transferred calibrations

After slope/bias correction on the receiving spectrometer, the transferred calibration equations became:

UK equation adjusted on USA instrument:

% moisture = 1.973 − 166.161 (D_{1914}) − 75.343(D_{1340});

USA equation adjusted on UK instrument:

% moisture = 2.106 − 98.339 (D_{1920}).

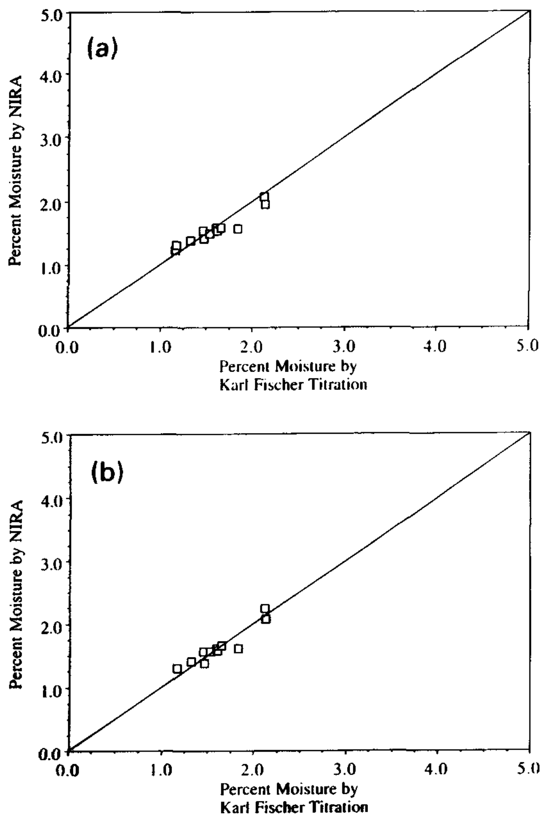

Figure 2
(a) UK test set results predicted using UK dual wavelength calibration. (b) UK test set results predicted using USA single wavelength calibration. (NIRA = Near-infrared reflectance analysis.)

Table 2
Summary statistics of NIR test set data

	Residuals (NIR-KF)			
	Bias		Accuracy	
Test set	Mean	SD	Mean	SD
(A) Using UK 2-wavelength calibration				
UK	−0.04	0.11	0.09	0.07
USA1	−0.01	0.22	0.19	0.10
USA2	−0.06	0.20	0.16	0.14
USA3	−0.01	0.23	0.16	0.16
USA3Q	−0.06	0.20	0.16	0.13
(B) Using USA 1-wavelength calibration				
UK	+0.02	0.11	0.09	0.06
USA1	0.00	0.24	0.20	0.13
USA2	−0.05	0.25	0.19	0.17
USA3	−0.04	0.24	0.16	0.17
USA3Q	−0.09	0.20	0.17	0.13
(C) Using USA 2-wavelength calibration				
UK	−0.03	0.17	0.14	0.08

UK is a UK test set scanned on the UK instrument; USA1, 2 and 3 are indepedent USA test sets scanned on the same instrument at that site; USA3Q is test set USA3 scanned on a second USA instrument.

Note: Bias takes into account the algebraic sign of the residual: accuracy ignores the sign.

These adjusted calibration equations were then tested using the local test set data scanned in at the receiving site, and NIR predictions were generated for comparison with the reference KF data. Residual data (NIR − KF values) are summarized in Table 2 for all between-site test sets, and a scatter plot of one test set, overlaid with the ideal correlation line, is shown in Fig. 2(b).

After forcibly generating a dual wavelength calibration equation from the USA calibration set, the equation was subjected to slope/bias correction versus the UK calibration set, which yielded the following equation:

$$\% \text{ moisture} = 2.063 - 92.547\,(D_{1920}) - 434.108\,(D_{1174}),$$

where 1174 nm was a software-selected wavelength that presumably corresponds to a C—H band. This equation was tested against the UK test set and the summary data are included in Table 2.

Precision study

Repeatability data covering eight determinations on each of six samples resulted in relative standard deviations ranging from 0.74 to 2.60% and 0.75 to 4.91% on each of two instruments. Agreement of the mean values for each sample was excellent between instruments, the differences averaging 0.02% w/w with a range of 0.0–0.8% w/w on mean results in the range 1.12–1.63% w/w.

Reproducibility data showed analyst-to-analyst variations in the range 0.01–0.09% w/w for 11 samples ranging in moisture content from 0.96 to 2.45% w/w.

Assessment of results

The data summarized in Table 2 demonstrate that excellent agreement was obtained between NIR predicted values and the KF reference values, even when calibrations have been transferred from another instrument, with correlation coefficients of up to 0.95 being achieved. Paired *t*-tests showed that in 10 of the eleven test set trials there was no significant difference between the NIR and KF results at the 95% level, and in the remaining set there was no significant difference at the 98% level. These NIR procedures are therefore very robust for the determination of moisture in this product, and have successfully accommodated differences between manufacturing sites, fill weights and glass vials. Precision data have demonstrated that the accuracy of the methods is supported by good repeatability and reproducibility.

Conclusions

The success of any NIR method is dependent upon the quality of the reference data used in the calibration. However, NIR has the potential in principle, to generate more accurate and precise data than KF titration since it avoids the need to open the vials and risk contamination from atmospheric moisture. In addition, NIR is rapid, convenient, non-destructive and non-invasive, and this work has demonstrated that such advantages can be exploited while generating reliable data. Approximately 40 samples can be analysed in 1-h, making the procedure ideal for a busy quality control laboratory.

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