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Suitability of near-infrared methods for the determination of moisture in a freeze-dried injection product containing different amounts of the active ingredient*

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Abstract: A near-infrared reflectance (NIR) method for determination of moisture in an experimental freeze-dried injection product was developed and validated. NIR spectra were collected through the bases of unopened product vials using a horizontal instrument accessory, before generating primary reference data on the same individual vials by Karl–Fischer titration. Data were collected for product containing different concentrations of the active ingredient in the same matrix. NIR calibrations were developed with second derivative spectral data using regression facilities within the NIR software, and validated using independent test sets. An assessment is given of the applicability of moisture calibrations developed on product at one active ingredient level to the prediction of moisture contents in the product containing a different concentration of active ingredient.

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

Introduction

Near-infrared (NIR) spectrometry is becoming an important technique for pharmaceutical analysis. The field has been reviewed [1, 2] and specific applications to the determination of water in solid dosage forms [3] and in lyophilized products [4] have been described.

This paper describes the development of NIR reflectance methods for the determination of moisture in two experimental freeze-dried injection products, as alternatives to the currently used Karl–Fischer (KF) titration procedures which are time-consuming and labour intensive and can be prone to error due to interference by atmospheric moisture.

NIR is well suited to the measurement of water since there are pronounced O—H overtone and combination bands 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 readily be taken through the walls of glass vials with minimal interference or spectral scatter from the glass itself.

The NIR methods were therefore developed by scanning the spectra of the intact freeze-dried solid through the bases of unopened product vials. NIR is essentially a secondary technique, and relies on calibrating the NIR response against reference analytical data generated on a calibration set of samples incorporating all the variations representative of real samples which will subsequently be analysed.

In the present work, NIR calibrations were set up, using KF titration reference data, for two closely related products. These each contained the same weight of the same excipient mix, but differing (low-level) amounts of the drug substance (0.5 and 1.5 mg vial^{-1} equivalent to ~0.2 and 0.6% w/w). It was of interest to explore the similarities between the calibrations for the two product strengths, since the moisture levels were known to be similar in each, and the small difference in the matrix might cause no perceptible differences in the NIR calibrations. Furthermore the investi-

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gation was extended to explore the robustness of the calibrations, covering product manufactured not only as separate batches but also in different production campaigns, and culminating in a combined calibration for use on both products.

Experimental

Apparatus

An NIRSystems 6500 model spectrometer was used, equipped with a horizontal set-up module (HSM) and NSAS software. KF titrations were carried out using a Metrohm E547 Automat titrator.

Reagents

Hydranal Composite 5 (Riedel de-Haen) KF reagent and Superpurity Methanol 205 (Romil Chemicals) were used in the KF determinations.

Samples

One hundred and two vials of the 0.5 mg product and 109 vials of 1.5 mg product were sampled from an extensive freeze-drier mapping study. The sample select facility within the NSAS software was used to select particular vials to cover 95% of the variation detected in the NIR spectra for each product. Thirty five vials and 38 vials were selected for the 0.5 and 1.5 mg products, respectively, and used to establish separate calibration sets: the remaining vials (67 for 0.5 mg and 71 for 1.5 mg product) were retained as independent test sets.

Further samples were taken from a later production campaign when three and four batches of 0.5 and 1.5 mg products, respectively, were manufactured for use in clinical trials. Totals of 15 and 20 vials, respectively, of the 0.5 and 1.5 mg products were collected, and used first as further independent tests for the existing calibrations, and subsequently in combination with samples from the first campaign to provide composite calibration and test sets to cover both products and both campaigns. Details of all sample sets are shown in Table 1.

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 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: 32 replicate scans 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.

Table 1
Water contents of calibration and test sets

			Water content (mg vial^{-1}) by KF		
Product	Sample set	No. in set	Mean	Range	% RSD
First production campaign (freeze-drier mapping)					
0.5 mg	All samples	102	2.92	2.06–4.94	18.9
	Calibration	35	3.03	2.17–4.94	22.1
	Test	67	2.87	2.06–4.55	17.2
1.5 mg	All samples	109	3.27	2.09–4.85	16.9
	Calibration	38	3.38	2.26–4.27	16.1
	Test	71	3.21	2.09–4.85	17.4
Second production campaign (clinical trials batches)					
0.5 mg	All samples	15	2.84	1.61–3.86	21.5
1.5 mg	All samples	20	3.09	1.46–3.86	21.4
Combined sets from first and second production campaigns					
0.5 and 1.5 mg	All samples	246	3.08	1.46–4.94	19.1
	Calibration	66	3.11	1.46–4.55	19.6
	Test	180	3.07	2.06–4.94	18.9

Data processing

All spectra were stored as electronic data files on the computer hard disk, and the NSAS software was used to append to these files the KF water contents for each sample as reference data. The zero-order NIR spectra were then 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 in the glass vial bases.

Several different calibration equations were then generated for each calibration set. Single wavelength calibrations were based on the well-defined O—H combination band at around 1900 nm; dual wavelength calibrations were based on the 1900 nm band and a software-selected band (1726, 2182 or 1974 nm depending on the sample set) which arises from sample matrix absorptions; and Partial Least Squares (PLS) regression calibrations used the 1140–2460 nm region.

The calibrations for each product were then tested using the independent test sets for that product for both production campaigns, before testing each calibration against the test sets for the other product strength. Finally, the combined calibration was tested using the combined independent test set.

Results and Discussion

Calibrations for separate products

The single and dual wavelength calibration equations for each product from the first production campaign were: (1) 0.5 mg product: % moisture = 1.617 − 377.811 (D_{1910}), % moisture = 3.223 − 360.413 (D_{1910}) + 631.225 (D_{1726}); (2) 1.5 mg product: % moisture = 2.358 − 230.761 (D_{1910}), % moisture = 1.073 − 50.264 (D_{1910}) + 111.007 (D_{2182}), 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.

PLS calibrations were developed for each product based on up to five PLS factors. There is a risk that the use of too many factors may lead to overfitting of the data (i.e. the calibration models noise as well as meaningful information), and therefore PLS work was carefully monitored.

There appeared to be only marginal differences between discrete wavelength calibrations and PLS. In all cases there was significant scatter in the data, with NIR and KF correlations and bias being noticeably worse for the 1.5 mg than for the 0.5 mg product. Predictions on the independent test sets followed similar patterns, although it was noted that PLS calibrations performed slightly better than discrete wavelength calibrations. The predictions for the 1.5 mg test set were again the worst, but this appeared at least in part to be due to clustering of samples in the centre of the range.

Cross-over studies

With only limited success for calibrations on each product strength, it was not unexpected to find that no greater success was achieved with attempts to use calibration equations for one product either for predicting results on the other or for predicting results on test samples from different production campaigns of the same strength product.

The reasons for the worse-than-expected results were sought, and close examination of the NIR spectra revealed wavelength shifts and changes in band shape, as illustrated in the second derivative plots of the 1900 nm water band in two samples shown in Fig. 1. These variations were observed as the drug:water ratio varied, either as a result of different water contents in one product, or as a result of similar water contents in products with different drug concentrations. It is therefore apparent that water is bound in different ways in the presence of different levels of active ingredient, presumably involving changing hydrogen bonding effects at varying water: drug ratios.

It is well established in NIR calibration work that successful calibrations can only be generated from samples representing all likely variations in composition and physical condition, and the inclusion of different batches from different production campaigns is a key factor here. For this reason, a combined calibration set appeared to offer the best potential for improved results.

Combined calibration set

In order to improve robustness and obtain a moisture calibration that would be applicable to both product strengths, the NIR spectral data files for all the samples from both production campaigns for both strength products were combined into one set and the NSAS

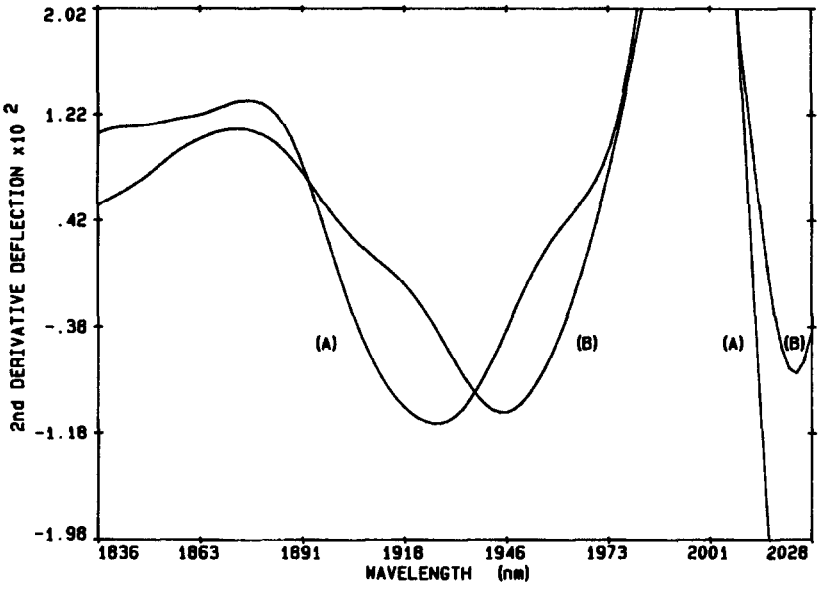

Figure 1
Expanded second derivative spectra showing changes in the 1900 nm water combination band for differing moisture:drug ratios. (A) Ratio 0.11, (B) ratio 0.39.

software sample select facility was used to extract a calibration set of samples which covered 95% of the spectral variation. Sixty six samples were selected, and the remaining 180 samples constituted the independent test set.

Single and dual wavelength calibrations were developed, and are described by

$$\% \text{ moisture} = 0.605 + 240.029\ (D_{1880}),$$

$$\% \text{ moisture} = 2.233 + 250.079\ (D_{1880}) - 159.393\ (D_{1974}).$$

PLS calibrations based on up to five PLS factors were also developed, and the five-factor PLS equation gave the best fit, as shown in the examples in Fig. 2. Results obtained on the combined test set are shown in terms of NIR − KF residual data in Table 2 and examples are presented graphically in Fig. 3.

Again PLS calibrations proved capable of performing slightly better than discrete wavelength calibrations, although the clustering of samples in the centre of the range is an unfortunate circumstance for all calibration tests since the full range is not tested adequately.

Assessment of results

This work has emphasized the need to incorporate all possible moisture concentration and manufacturing process variables in order to achieve a robust calibration. The final combined product calibration set gave reasonable NIR predictions for both products, despite indications that water is bound in a variety of ways in these products. Paired *t*-tests on the combined test set data showed that there was no significant difference between NIR and KF data at the 95% levels for the single wavelength or five-factor PLS predictions, but that there was a significant difference at the 95% level for the dual wavelength predictions. Examination of the distributions of the data sets showed that the dual wavelength set had a lower mean value and was skewed when compared with the KF data or the other two NIR sets.

The combined-set calibrations are currently being tested against samples from a third production campaign.

Conclusions

By combining all available samples it has proved possible to develop reasonable NIR moisture methods to cover both strengths of this product. This combined approach improves the robustness and predictive ability when compared with individual product calibrations. However, the accuracy and precision are not good enough to allow the use of the current method in anything but a screening role.

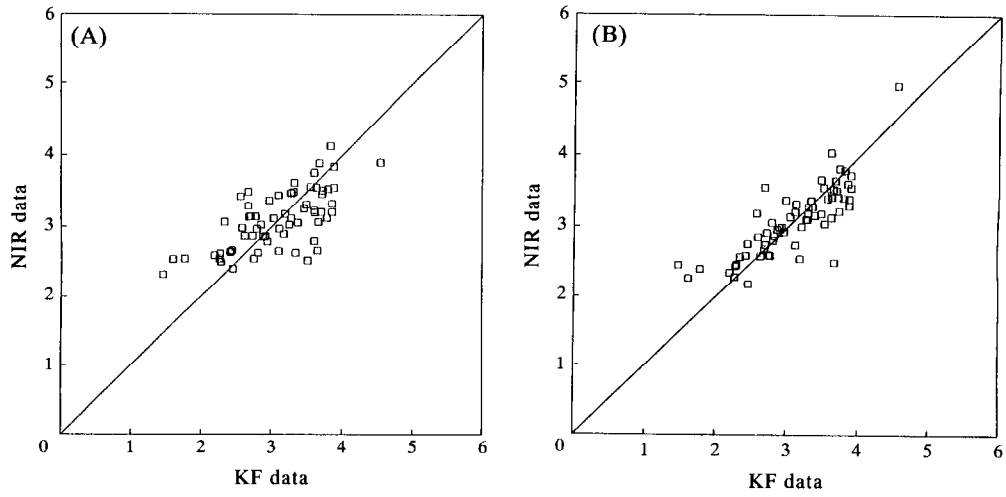

Figure 2
Calibration plots for moisture in combined 0.5 and 1.5 mg product sample sets. (A) Dual wavelength calibration, (B) five-factor PLS calibration.

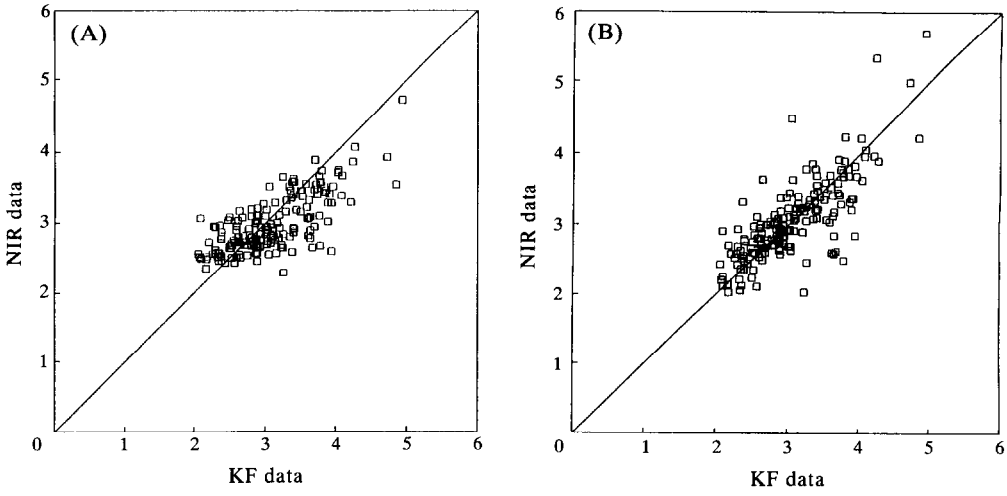

Figure 3
NIR predictions for moisture in independent test set of combined 0.5 and 1.5 mg samples, (A) using dual wavelength calibration, (B) using five-factor PLS calibration.

Table 2
Summary statistics for NIR data on combined test set using combined calibration sets

	Residuals, NIR − KF (mg vial^{-1})			
	Bias		Accuracy	
Calibration equation	Mean	SD	Mean	SD
1λ	−0.01	0.48	0.38	0.29
2λ	−0.09	0.38	0.31	0.24
PLS-1F	−0.01	0.50	0.38	0.31
PLS-3F	+0.05	0.40	0.29	0.27
PLS-5F	−0.01	0.35	0.25	0.25

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

Variations in hydrogen bonding effects at different water:drug ratios in these two products appear to contribute to poorer performance of these NIR methods when compared with those developed recently for another freeze-dried injection product [5]. The best correlation between NIR and KF data achieved in this work was 0.81, compared to 0.95 for the work reported in ref. 5.

Further work is in progress which may facilitate improvements to this method by incorporating further samples from other production campaigns and by optimizing the wavelength regions used.

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