

Standardisation of factory online cane analysis systems for the NIR measurement of brix and pol in juice

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Introduction

Highly NIR absorbent materials such as sugarcane present a considerable challenge for chemometricians in the form of long term stability of calibrations. The primary factors affecting calibration stability are sample variation, which can be handled with the inclusion of new samples into the calibration process, and instrument variation, which is more difficult to incorporate in the calibration process for highly absorbent materials, due to non-linear instrument (absorbance) measurement.

One solution to the instrument variation problem is to include multiple instruments within the calibration set. However, most calibrations become trained on the instruments rather than incorporating the variation into the resulting equations. This is evident when a multiple instrument calibration fails to predict adequately on a previously unseen instrument. The training effect is largely due to an insufficient number of instruments present in the calibration process, which can be circumvented by simply increasing the number of instruments into the calibration. Ultimately, adding instrument variation is not feasible due to the large number of instruments (and expense) required. In cases where sample variation is also necessary within each instrument, this renders the number of samples required for calibration enormous! An alternative solution to instrument variation is standardisation.

Standardisation of NIR instruments has been common practice in areas involving highly reflective samples like grains and pharmaceutical products. NIR instruments operate almost linearly in the highly reflective absorbance region ($A < 1$), which has facilitated calibration transfer between instruments with minimal alterations to calibrations, with a simple bias adjustment being all that is required in most situations. However, up until now there has been no satisfactory method to standardise NIR instruments for highly absorbing products.

Highly absorbing products are characteristically measured in the non-linear region of NIR instruments. The non-linear absorbance of these materials in this region is highly instrument specific, and has eluded linear methods of instrument calibration transfer. This paper describes a

method for measuring NIR instrument non-linear responses on highly absorbing materials, and their subsequent use to derive standardised spectra and resultant standardised calibrations.

Materials and methods

Six networked online Cane Analysis System (CAS) instruments (FOSS 5000 Direct Light models) were benchmarked for their absorbance response by measuring a set of custom made, NIST traceable, photometric standards of approximately 3%, 5%, 10%, 40% and 99% reflectance (Figure 1).

The photometric standards were custom made to fit the entire sampling window of the CAS instruments and were measured *in situ* at each of the sugar mill installation sites.

Absorbance spectra were recorded for the set of photometric standards from 1100 nm to 2498 nm in transmission mode. Due to signal deterioration caused by transmission via an optic fibre bundle, spectra were effectively recorded from 1100 nm to 2100 nm. The spectra of the standards were used to construct a non-linear mapping between each of the instruments and a virtual master instrument (the known response of the photometric standards).

Sugarcane calibrations were developed using both the non-standardised spectra and standardised spectra to assess the usefulness of the standardisation procedure. Approximately three thousand spectra were collected, with five hundred samples taken from each CAS instrument. Five of these sites were used for calibration development, while the sixth was treated as an independent validation set. Calibration of the data from five sites for Brix and pol in juice was performed using

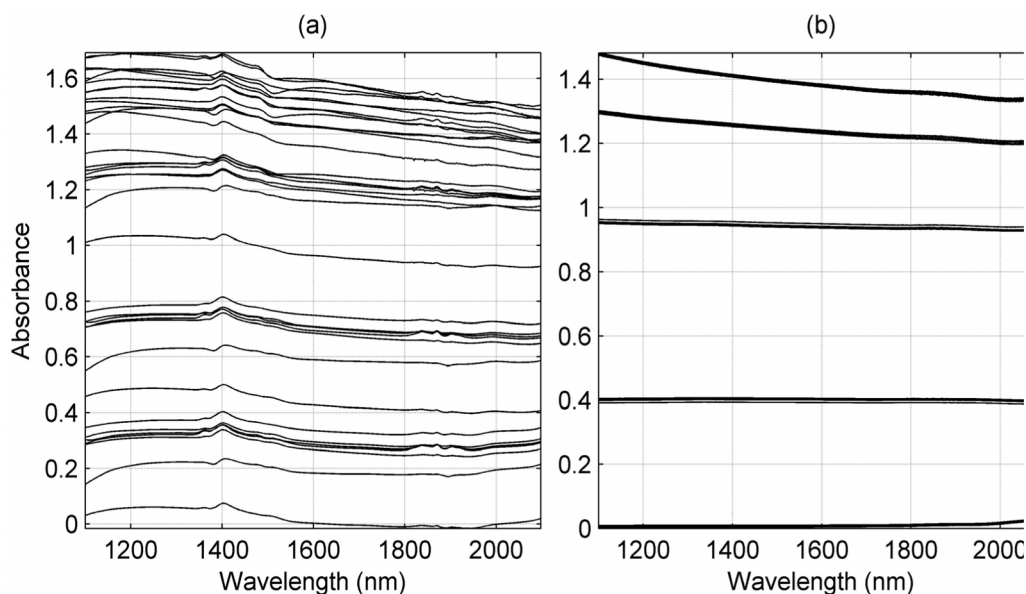


Figure 1. Instrument photometric absorbencies for the six CAS instruments (a) raw (b) standardised.

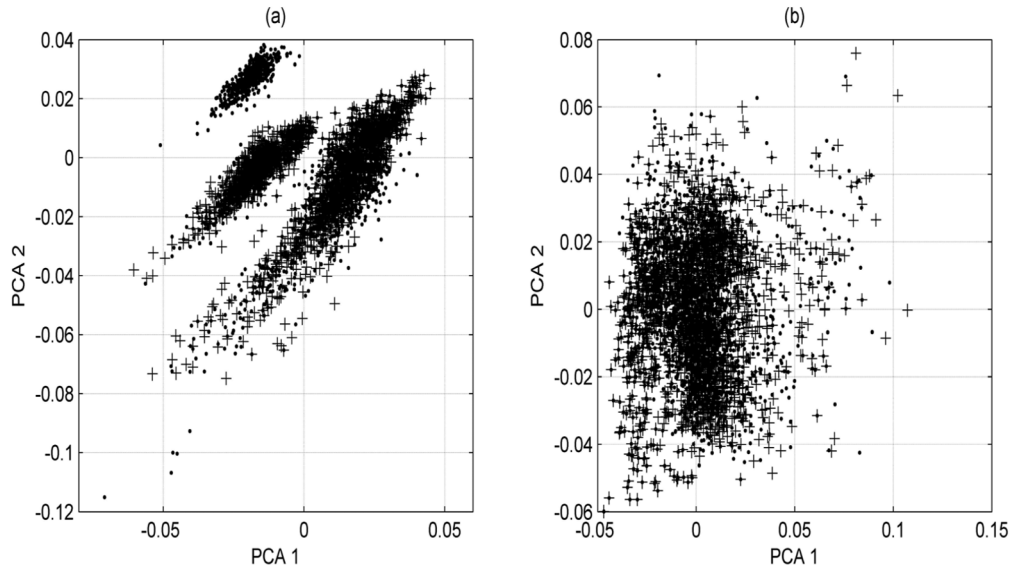


Figure 2. PCA 1 Vs PCA 2 for the calibration (•) and validation (+) data for (a) normal spectra and (b) standardised spectra.

WinISI II, and calibration equations were built using SNV transformed, first derivative (1,4,4,1) spectra via the MPLS regression option.

Results

Instrument variation was largely corrected by the standardisation algorithm with the photometric standard set for all instruments being representative of the virtual master after standardisation (Figure 1). Standardisation of the calibration spectra resulted in an obvious reduction in instrument to instrument variability (Figure 1 & 2), which is readily observed in the first two principal components of the calibration set.

Table 1. Calibration and validation statistics for Brix and pol in juice calibrations for non-standardised and standardised spectra.

Constituent	Standardised	Calibration set			Validation set				
		<i>N</i>	<i>R</i> ²	<i>SEC</i>	<i>N</i>	<i>R</i> ²	<i>SEP(C)</i>	Slope	Bias
Brix	No	2454	0.956	0.294	689	0.906	0.475	1.112	0.07
Brix	Yes	2482	0.962	0.277	1592	0.965	0.274	0.977	0.003
Pol	No	2478	0.957	0.299	1519	0.952	0.322	0.986	−0.162
Pol	Yes	2490	0.964	0.276	1602	0.965	0.272	0.988	0.027

Table 2. Number of outliers for the calibration set ($n = 2500$) and validation set ($n = 1652$).

Constituent	Standardised	Calibration	Validation
		% outliers	% outliers
Brix	No	1.84	58.29
Brix	Yes	0.72	3.63
Pol	No	0.88	8.05
Pol	Yes	0.4	3.02

Variations in the non-standardised spectra are dominated by the discrete instruments and a cane supply effect (Figure 2).

Two of the instruments are installed at sugar mills which process more burnt cane than the remaining three mills in the calibration set. Variability in the standardised spectra is predominantly related to differences in cane supply.

Validation data from the sixth mill exhibited similar grouping to both the non-standardised and standardised calibration data (Figure 2). Therefore it was expected that the validation data would fit nicely into the standardised equations without exhibiting many outliers or requiring equation adjustments.

Equation statistics for both the non-standardised and standardised calibration sets were very similar, with the standardised spectra producing slightly better performance (Table 1).

Both sets produced acceptable regression statistics for their respective calibration data. Outlier deletion was used during the calibration exercise for both sets which accounts for the reduced number of calibration samples reported (Tables 1 and 2).

Validation of the non-standardised spectra with data from the sixth mill gave poor results for the Brix equation with a substantial increase in $SEP(C)$ (Table 1) and many samples being rejected as outliers (Table 2). The equation for pol in juice for the non-standardised spectra resulted in adequate performance with a minor change in bias and $SEP(C)$ (Table 2).

Standardised equation validation performance for both Brix and pol in juice was as good as the calibration data (Table 1), and also led to a decrease in the percentage of outliers observed (Table 2).

Conclusion

Standardisation procedures for the NIR analysis of high moisture samples using traceable photometric standards produces standardised calibrations which validate more favourably than their global calibration counterparts. The standardised equations required no adjustment when applied to new instruments and accurately predicted a greater proportion of samples for both calibration and validation data sets.

By removing instrument differences, spectral libraries can be reduced in size, leading to a cost reduction for developing future calibrations. Further, the standardisation technique corrects

for non-linear instrument characteristics over the entire absorbance range, rendering the method applicable to both high and low absorbing products.

High absorbance standardisation also resolves the challenging issue of calibration transfer between instruments of the same type. It is possible that similar methods could be used to transfer calibrations developed on one instrument platform to an entirely different platform.

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