

Validating a network of near infrared spectroscopy instruments for mixed feed analysis

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Introduction

In 2001 near infrared (NIR) calibrations for the analysis of mixed feed samples were built. The used calibration database consisted of a consolidation of four databases, each one compiled on a different NIR instrument. Since two of these instruments were no longer available, a standardisation of the spectra could not be performed.

The calibrations were installed on five different instruments. During 2002 validation samples were collected.

In this paper the validation of the performance of the calibrations on the different instruments is discussed.

Material and methods

Database

The calibration database contained 530 near infrared spectral data originating from four different BRAN & LUEBBE InfraAlyzer 500 (IA500) monochromators (instrument A, B, C and D). Calibration samples consisted of mixed feed samples originating from different geographical locations and collected over a three-year period (1999–2001). The samples included ruminant, poultry and pig feed. Table 1 illustrates the sample representation.

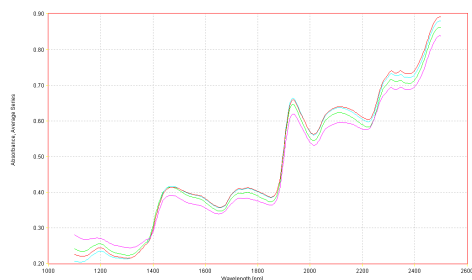
Table 1. Calibration sample representation

Instrument	N	Year	N ruminant feed	N pig feed	N poultry feed
BRAN&LUEBBE IA500 A	130	1999	130	—	—
BRAN&LUEBBE IA500 B	43	1999–2000	12	31	—
BRAN&LUEBBE IA500 C	178	2001	60	105	13
BRAN&LUEBBE IA500 D	179	2001	58	107	14
Total	530		260	243	27

Samples were ground through a 0.5 mm screen and scanned from 1100 nm to 2500 nm every 4 nm, using BRAN&LUEBBE IA500 monochromator instrumentation equipped with a rotating sample cup. The average absorbance-spectrum of the calibration databases is shown in Figure 1(a).

The samples were analysed in the lab according to the EC methods. Most samples were analysed for moisture, crude protein, crude fibre, crude ash, crude fat, sugar and starch.

(a)



(b)

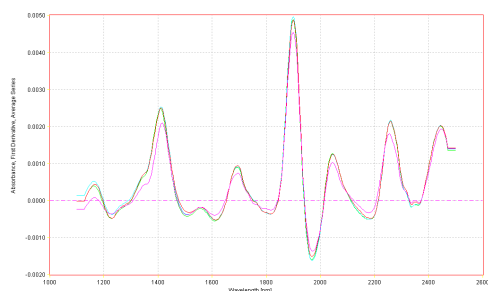


Figure 1. The average spectrum of the calibration databases. (a) absorbance-spectra and (b) absorbance-spectra after first derivative pre-treatment.

Calibration

Since two of the instruments on which data were collected, were no longer available, a standardisation of the spectra could not be performed. Therefore the spectra of the different instruments were consolidated in one database and the first derivative pre-treatment was used to minimise the scattering effects of instrument differences and particle size. The study of the average absorbance-spectra of the individual calibration databases of instrument A, B, C and D after the first derivative pre-treatment [Figure 1(b)], revealed the high similarity of the BRAN&LUEBBE instruments.

The Sesame software was used to process the data and develop chemometric models. Calibration was performed using the PLSR model. The optimum number of PLS factors was determined by cross-validation. During cross-validation, one calibration sample at a time was temporarily removed from the calibration-set and used for prediction. Performance of the calibration was reported as the standard error of estimate (*SEE*), the standard error of cross-validation (*SECV*), the multiple correlation coefficient (*R*) and the ratio of *SD* to *SEE* (*SD/SEE*) as presented in Table 2.

Table 2. Calibration performance.

parameter	Lab			NIR				<i>SD / SEE</i>
	Mean (%)	<i>SD</i> (%)	Range (%)	<i>SEE</i> (%)	<i>SECV</i> (%)	<i>R</i>	<i>N</i>	
moisture	11.26	1.27	6.7–14.23	0.39	0.41	0.95	370	3.3
crude protein	17.80	2.31	12.01–28.61	0.59	0.62	0.97	402	3.9
crude fibre	7.43	3.44	1.86–15.05	0.88	0.93	0.97	368	3.9
crude ash	6.34	1.58	2.8–10.8	0.65	0.7	0.91	380	2.4
crude fat	4.74	1.88	2.39–13.92	0.31	0.32	0.99	158	6.1
sugar	6	2.43	2.99–14.28	0.8	0.84	0.95	212	3.0
starch	34.99	8.14	17.39–55.9	1.63	1.72	0.98	241	5.0

Validation

The calibrations were validated with an independent set of feed samples collected during 2002 on five different BRAN & LUEBBE IA500 instruments (instrument C, D, E, F and G). The calibration database contained spectra of only two of them (instrument C and D) while no spectra of instrument E, F and G was used in the calibration. Table 3 illustrates the sample representation.

Table 3. Validation sample representation.

Instrument	N	Year	N—ruminant feed	N—pig feed	N—poultry feed
BRAN&LUEBBE IA500 C	397	2002	300	46	51
BRAN&LUEBBE IA500 D	328	2002	31	230	67
BRAN&LUEBBE IA500 E	255	2002	92	110	53
BRAN&LUEBBE IA500 F	205	2002	50	107	48
BRAN&LUEBBE IA500 G	170	2002	53	117	
Total	1185		526	610	219

Model performance was reported as the standard error of prediction (*SEP*) calculated for every parameter and every instrument.

To validate the performance of the calibration, the *SEP* to *SECV* ratio (*SEP/SECV*) was calculated for every parameter and every instrument. If the calibration is robust and easily transferable to new instruments, similar ratios with a value close to 1 were expected for each parameter on the different instruments. Table 4 illustrates the *SEP/SECV* ratios for the different instruments.

Table 4. *SEP/SECV* ratios for the different instruments.

	instrument C	instrument D	instrument E	instrument F	average C–F	instrument G	average all
parameter	%	%	%	%		%	
moisture	1.17	1.24	1.17	1.05	1.16	1.95	1.32
protein	1.15	1.24	0.94	1.00	1.08	1.65	1.19
fibre	0.70	0.97	0.87	0.89	0.86	1.37	0.96
ash	1.09	0.77	0.89	0.96	0.93	1.30	1.00
fat	1.28	1.56	1.78	2.34	1.74	1.78	1.75
sugar	1.10	1.12	1.02	1.12	1.09	—	1.09
starch	1.43	1.17	0.95	0.92	1.12	1.97	1.29

Results and discussion

For instruments C, D, E and F most of the *SEP/SECV* ratios were close to 1 indicating that the statistical performance of the validation dataset was better (*SEP/SECV* ratio <1) or worse ($1 < \text{SEP/SECV} < 1.5$) than the statistical performance of the calibration dataset. For these instruments the ratios for one parameter were also very similar indicating that the calibration was equally performing on the different instruments. Only the crude fat calibration has larger *SEP/SECV* ratios (>1.5) for instrument D, E and F.

The *SEP/SECV* ratios for instrument G were always >1 and mostly >1.5. This poor performance of the calibrations on instrument G could be explained by the bad technical performance of the

instrument (Reference Energy measurement <150002). Figure 2 demonstrates the effect of this technical problem on the measured absorbance-spectra.

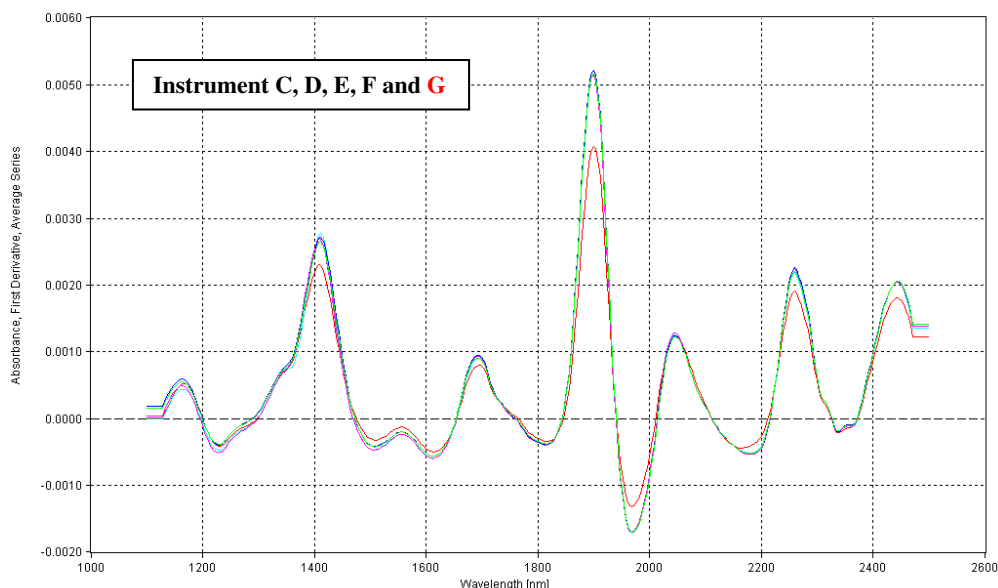


Figure 2. The average spectrum of the validation databases after first derivative pre-treatment.

Conclusions

Validation statistics

SEP/SECV ratios are very useful in the evaluation of the calibration performance on an independent validation dataset. An equal or better performance of the calibration on the independent validation dataset, will result in a *SEP/SECV* ratio <1. However, if a new calibration would equally increase the *SECV* and *SEP* value, the same *SEP/SECV* ratio would be found. The conclusion would be drawn that the calibration was performing well on the validation dataset but the calibration statistics themselves (*SEE* and *SECV*) would have increased. This demonstrates that the *SEP/SECV* ratio gives no information on the performance of the calibration in relation to the laboratory and makes the evaluation over time difficult. Therefore the need for an additional, fixed reference is high. No satisfactory additional reference is found at this time.

Calibration transfer

Because of the high similarity in the *SEP/SECV* ratios for each parameter for all technically well performing instruments, the conclusion can be drawn that the lack of a standardization file does not have a negative effect on the calibration performance on new instruments. Robust, easily transferable calibrations can be built by the consolidation of databases of different instruments. Even the amount of samples with an *H* value >3 was similar for all five instruments which confirms the high similarity between the BRAN&LUEBBE instruments. The calibration is transferable, even to instruments of which no spectra were included in the calibration database and with the lack of a standardisation file.

The *SEP/SECV* ratios for all well performing instruments, indicate an equal performance of the calibration on the validation dataset with the exception of the crude fat calibration. The low amount of calibration samples for the crude fat calibration may be the cause for this.

The technical performance of the instruments has an important influence on the measured spectra (see Figure 2) and therefore also on the validation performance. High care and consideration has to be taken on a regular, profound validation of the instruments' performance. Additional tests to the ones described by the supplier may be needed to perform these validations.

References

1. BIPEA. 13-Aliments des animaux. Résultats de Septembre 2002, n°338.
2. BRAN+LUEBBE. InfraAlyzer[®] 500, Near Infrared Reflectance Analyser. *Operation Manual*, Chapter 5, p.23 (November 1994).