Near infrared spectroscopy as a tool to control the results of chemical analyses performed as single determinations

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Introduction

In analytical laboratories chemical analyses are normally performed in duplicate in order to validate the results. When the difference between two duplicates is greater than a critical value [1], the analysis has to be repeated one more time to check the value.

This procedure requires a large number of individual measurements for large analytical series. In some laboratories, analyses are performed as single determinations using a control sample for each series. This procedure reduces the number of individual assays but it does not allow for control of all the individual results increasing the risk of error.

In the animal feed and forage laboratory of CIRAD, analyses are usually performed in duplicate, even for large series (hundreds to thousands) of samples. It was decided to investigate the potential of near infrared (NIR) spectroscopy for the control of analyses performed as single determinations, while maintaining the use of reference methods and ensuring a high standard of quality control and reliability.

Principles of error detection

The procedure chosen was to compare the analytical result to the NIR spectroscopy prediction provided by an equation of known SEP (standard error of prediction, [2]). If the difference is greater than a critical value then the result is discarded and the assay is rerun.

The gain in accuracy provided by this strategy is difficult to calculate directly because the corrected values do no longer follow a normal Gaussian distribution.

A numeric simulation was performed using 10,000 independent values to predict the improvement in accuracy obtained by a control by NIR spectroscopy. Obtained by statistical adjustments of simulations, equation 1 describes this improvement if the level is p = 5% (elimination of outliers when |x-y| >1.96 x SEP).

$$s_{corr} = s - 0.12 \ \frac{s^3}{SEP^2} \qquad \text{Equation 1}$$

s_{corr} : Standard deviation of errors for the result after elimination of outliers detected by NIR spectroscopy
s : Standard deviation of errors for the analysis
SEP : Accuracy of the NIR spectroscopy calibration

In a case where another level (L) other than 1.96 is chosen for outlier elimination, the relationship may be described by equation 2.

$$s_{corr} = s - 0.085\,(3 - L)^2 \ \frac{s^3}{SEP^2} \qquad \text{Equation 2}$$

These approximate relationships were established under the hypothesis that initial errors on both laboratory measurement and NIR spectroscopy prediction follow a normal distribution.

These equations only concern the detection of outliers of the normal error distribution.

In practice NIR spectroscopy prediction can also be used to track laboratory mistakes and sampling problems. These errors generally do not follow identifiable probability laws. Therefore the approach of using NIR spectrosopy for that purpose is more empirical.

Example of application

An application of this analytical strategy was performed on a real dataset. The classical duplicate analysis (strategy S1) was compared to the use of NIR spectroscopy to control single analyses (strategy S2).

Fifty poultry feed samples were analyzed for fibre content (WICW, water-insoluble cell walls [3]). Their NIR spectra were collected in duplicate (two different cup fillings) in diffuse reflectance mode on a Foss NIRSystems 6500 spectrometer (Foss NIRSystems, Silver Spring, MD, USA) and averaged.

Analysis strategy S1: classical duplicate analysis

Analyses were performed in duplicate. Analyses were re-run if their difference was greater than d = 1.15%. This level was calculated from repeatability of analysis (noted r) with a 95% probability level [4] with the formula:

$$d = 1.96 \sqrt{2}\, r \qquad \text{Equation 3}$$

d: difference between repeat analyses
r: repeatability coefficient

Analysis strategy S2: verification by NIR spectroscopy

Laboratory results obtained with single analysis were checked against an existing prediction equation (PLS regression; R^2 = 0.98; SECV = 1.43%. See Table 1). Analyses were re-run if measurement and prediction differed by more than 1.15%. The elimination level was the same as for S1 (instead of being based on SEP), in order to strengthen error detection. Analysis was run a third time if the two previous assays differed by more than 1.15%.

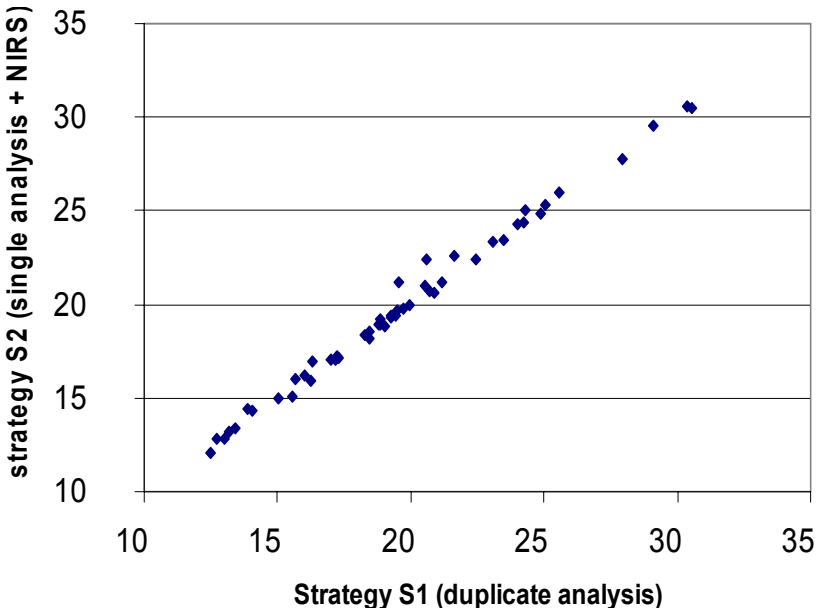

Figure 1. Comparison of predictions from strategy S1 (duplicate analysis) and strategy S2 (single analysis + NIR spectroscopy).

Table 1. Calibration model: Cell walls in poultry feeds.

Constituent	Population		Calibration statistics			
	Mean	SD	SEC	R^2	SECV	RDP
Cell walls (%)	20.8	5.7	0.89	0.98	1.43	4.0

SD: standard deviation of the population
SEC: standard error of calibration
R^2: coefficient of determination of calibration
SECV: standard error of cross-validation
RPD: ratio of performqnce to deviation ($SD.SECV^{-1}$)

The agreement between the results obtained by the two strategies was R^2=0.996 (Figure 1: intercept = 0.22, slope = 1.01). All outlier values were detected by NIR spectroscopy. The number of samples to be re-analysed was 12 for S1 and 17 (ten in duplicate and seven in triplicate) for S2, leading to a total number of assays of 112 for S1 and 74 for S2.

Discussion

This analytical procedure was repeated three times on the same poultry feed samples. In one case, one outlier was not detected, but this happened in both strategies S1 and S2.

One problem of the verification with NIR spectroscopy would occur if the prediction of the new dataset performed with the equation is biased, such is if the existing calibration equation fails to predict correctly the samples. To solve this, it is possible to include the new samples (analyzed as single determinations) in the calibration set and re-compute calibration process. The advantage is that the new calibration equation is adapted to the new series of samples. The problem is the risk that analytical errors can be included in calibration and therefore not detected properly. This strategy (S3) should therefore be used only for calibrations based on large datasets, because in that case the outliers have a lower weight on the calibration building.

In the present study, we considered that the analytical errors come from reference method only. The underlying hypothesis is that the variability of NIR spectroscopic measurements is negligible compared to the reference method. This is generally the case in our laboratory, because we work with ground samples and we always average spectra from two different cup fillings. The situation could however, be different for NIR spectroscopic measurements done on raw samples with higher spectra acquisition variability.

Conclusions

The conclusion of this study is that the validation of single measurements by an existing NIR spectroscopic equation for large series of samples is a powerful tool to reduce the number of measurements in the laboratories, while maintaining a systematic control of results and the use of the official analytical method. Number of individual analyses is decreased by 30 to 50% which is considerable in routine. Besides, we have noticed several times that calibration equations developed using single determinations have SEP or SECV values close to the ones developed using duplicate analyses. Building new calibration with samples analysed only once is therefore more efficient because, for the same analytical cost, the calibration can be based on more samples with. more variability. The procedure is also interesting for calibration development because it can identify prediction outliers for which the reference value is confirmed. These are of particular interest when developing robust calibration equations.

The experience with this strategy for routine analysis shows that the errors detected by NIR spectroscopic verification are more often due to analytical problems and mistakes than outliers from the normal distribution of analytical errors. Furthermore, in addition to being a tool for analysis

control NIR spectroscopy can also identify spectral outliers (non-typical samples), which can sometimes be useful information for the client.

This strategy is still efficient and useful even if the accuracy of the calibration equation is not sufficient for routine prediction. In other cases the quality of NIR spectroscopic predictions can get close to the precision of the reference laboratory (and even be better [5]) and NIR spectroscopic predictions can be used instead of reference values (if reference analysis is not explicitly required). In that case the problem is opposite since laboratory values are used to check NIR spectroscopic predictions.

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