


RESEARCH ARTICLE

Methodology adjusting for least squares regression slope in the application of multiplicative scatter correction to near-infrared spectra of forage feed samples

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Abstract

Scatter corrections are commonly applied to refine near-infrared (NIR) spectra. The aim of this study is to assess the impact of measurement errors when using ordinary least squares (OLS) for multiplicative scatter correction (MSC). Any measurement errors attached to the set-mean spectrum may attenuate the OLS slope and that in turn will affect the estimate of the intercept and the adjustment of the spectra when using MSC methods to mitigate scattering. A corrected least squares slope may be used instead to prevent this problem, although the impact of this approach on the final outcome will depend on the relative size of the measurement errors in the individual spectra and the set-mean spectrum. The errors-in-variables or type II regression model (also known as Deming regression) and its special cases, major axis (MA) and reduced major axis (RMA), are discussed and illustrated. The extent of OLS slope bias or attenuation is demonstrated as is the resulting MSC spectral distortion. Further modification to the MSC transformation method is also suggested. The influence of scattering correction (by MSC, standard normal variate (SNV) and detrending) and of using the maximum likelihood estimate of the slope for MSC on the prediction of chemical composition of Lucerne herbage from NIR spectra was assessed. The predictive performance was slightly improved by the use of scattering corrections with fairly minor differences among methods. Nonetheless, it seems well worth considering the use of type II regression models for assessing MSC application aiming at improving the goodness of prediction from NIR spectra.

KEYWORDS

errors-in-variables, measurement error models, multiplicative signal correction, near-infrared spectroscopy, slope attenuation, type II regression

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1 | INTRODUCTION

Near-infrared (NIR) spectroscopy is a rapid, non-destructive, robust, and accurate method with multiple applications in the fields of agronomy and food and feed sciences to assess chemical, physical, mechanical and anatomical properties of food and feed materials, such as grains and grain products, fruit and vegetables, edible oils, animal protein (meat, fishery or dairy products) and others.¹⁻³ NIR spectroscopy has been also used to evaluate soil properties in precision agriculture.^{4,5} This methodology has been used mostly to determine proximate chemical composition of agricultural products.

Each NIR spectrum is comprised of two reflection components, namely diffuse and specular. The diffuse reflection is the result of characteristic absorptions once radiation has penetrated into the particles of the sample, and thus depends on the biological nature and chemical composition of the substrate.⁶ NIR spectra are normally plotted on the logarithmic (base e) scale as $\log_e(1/R)$, where R is the diffuse reflectance. However, the observed spectrum is also related to the physical characteristics of the sample,^{7,8} because the incident radiation will interact with attributes such as particle size and shape, porosity, moisture, sample compaction and packing density, affecting the measured reflectance plots. The consequences of these effects are that NIR spectra often exhibit baseline shifts and that NIR calibration sets will entail some degree of multicollinearity.^{9,10} Aiming at decreasing the impact of the baseline shifts and mitigating the effects of multicollinearity, spectral pre-treatments or transformations are commonly applied prior to calibration modelling.¹¹⁻¹³ The most extensively used spectral pre-treatments are multiplicative scatter (or signal) correction (MSC),⁸ standard normal variate (SNV)¹⁴ and detrending to remove the linear and quadratic trends of each spectrum.¹⁴ Spectral scales will be specific to the transformations applied,¹⁰ either alone or in combination with derivatives and/or smoothing.

The SNV correction is applied by scaling each mean-centred spectrum

$$y_{\text{SNV},i} = \frac{(y_i - \bar{y}_i)}{\sigma_i}$$

where y_i is any observed value within the individual i -th spectrum, \bar{y}_i and σ_i are the average and the standard deviation, respectively, of the values in the sample spectrum to be corrected and $y_{\text{SNV},i}$ is the SNV corrected value.^{14,15} Thus, only the values included in each spectrum are used for the SNV correction. Each corrected spectrum has zero mean and variance equal to one, and is independent of both original linear scale and sample set characteristics.

To the contrary, MSC requires a reference spectrum, ideally free of any scattering.⁸ The underlying principle of MSC is usually presented as

$$y_{ij} = y_{ij}^o + \delta_{ij} = y_{ij}^o + (\alpha_i + \beta_i' y_{ij}^o + \varepsilon_{ij}) = \alpha_i + (1 + \beta_i') y_{ij}^o + \varepsilon_{ij} = \alpha_i + \beta_i y_{ij}^o + \varepsilon_{ij}$$

where, y_{ij} is the measured signal for sample i at wavelength j , y_{ij}^o is the 'true' (reference) signal, δ_{ij} is the deviation of y_{ij} from y_{ij}^o , α_i is the random offset noise for sample i , β_i is the random multiplicative noise for sample i , and ε_{ij} is the residual noise, normally assumed to be random and independent. The goal of MSC is to estimate α_i and β_i through a regression process and subsequently correct the signal to mitigate their effect. As the 'true' signal is unknown, the set-mean spectrum (y_m) has been most commonly used as the reference for MSC. The relationship between the individual (y_i) and the set-mean (y_m) spectra is necessarily linear, because both have a similar shape over the NIR wavelength range.

When applying MSC to a set of n spectra, it is necessary to estimate the slope ($\hat{\beta}_i$) between the i -th spectrum (y_i) and set-mean spectrum (y_m). Regression coefficients (intercept α_i and slope β_i) for application of MSC are usually estimated by ordinary least squares (OLS) by fitting the linear function

$$y_i = \alpha_i + \beta_i y_m + \varepsilon_i$$

where

$$\hat{\alpha}_i = \bar{y}_i - \hat{\beta}_i \bar{y}_m$$

This regression defines fitted or smoothed values of the individual spectrum as predicted by the set-mean spectrum and as a result some of the small spectral fluctuations are removed. The MSC corrected spectrum ($y_{\text{MSC},i}$) is then calculated as^{8,9}

$$y_{\text{MSC},i} = (y_i - \hat{\alpha}_i) / \hat{\beta}_i \quad (1)$$

or, substituting for $\hat{\alpha}_i$

$$y_{\text{MSC},i} = (y_i - \bar{y}_i) / \hat{\beta}_i + \bar{y}_m \quad (2)$$

Here, $(y_i - \bar{y}_i)$ represents a self-centred NIR spectrum, $1/\hat{\beta}_i$ a scattering reduction factor (or multiplicative constant) and the set mean average (\bar{y}_m) is the common offset.¹⁰ Effectively, an estimate of the set-mean spectrum ($y_{\text{MSC},i}$) is obtained using self-centred individual spectrum signals. The MSC calculation uses inverse interpolation to derive corrected spectra considering that the factor $(y_i - \hat{\alpha}_i) / \hat{\beta}_i$ is a ratio of dependent normal variables and follows a Fieller–Hinkley distribution.^{16,17} The inverse interpolation step in the MSC procedure gives ‘smoothed’ set-mean spectrum estimates, which again are net of small spectral variations.

Dhanao et al.⁹ showed that MSC and SNV spectra are linearly related as

$$y_{\text{MSC},i} = y_{\text{SNV},i} \frac{\sigma_m}{r_{i,m}} + \bar{y}_m$$

In this equation, the ordinate intercept equals \bar{y}_m and the slope equals $\sigma_m/r_{i,m}$, where σ_m is the standard deviation of the set-mean spectrum and $r_{i,m}$ the correlation coefficient between the i -th and the set-mean spectrum (expected to be close to their upper limit of 1 in most cases). Noticeably, \bar{y}_m and σ_m are unique to the set of spectra used for the MSC.

Two refinements to the MSC pre-treatment have been proposed, namely (a) extended multiplicative signal correction to eliminate largely uncontrolled path length or scattering effects¹⁸ and (b) signal interference subtraction to remove some known spectral interferences. Pedersen et al.¹⁹ derived a reverse procedure, an MSC-like spectral transformation with extended inverted signal correction (ISC). In its simplest form, ISC involves regression of the set-mean spectrum on the individual spectrum, that is

$$y_m = \alpha_{\text{ISC},i} + \beta_{\text{ISC},i} y_i + \varepsilon_i$$

with

$$\hat{\alpha}_{\text{ISC},i} = \bar{y}_m - \hat{\beta}_{\text{ISC},i} \bar{y}_i$$

After substitution and simplifying

$$y_{\text{ISC},i} = (y_i - \bar{y}_i) \hat{\beta}_{\text{ISC},i} + \bar{y}_m$$

In this calculation, there may be distributional consequences because there is a product of dependent normal variables.²⁰ The scatter reduction factor is $\hat{\beta}_{\text{ISC},i}$,²¹ with

$$r_{i,m}^2 = \hat{\beta}_{\text{MSC},i} \times \hat{\beta}_{\text{ISC},i}$$

The difference between MSC and ISC when OLS is applied in each case will be

$$y_{\text{MSC},i} - y_{\text{ISC},i} = (y_i - \bar{y}_i) \frac{\hat{\sigma}_m}{\hat{\sigma}_i} \left(\frac{1 - r_{i,m}^2}{r_{i,m}} \right)$$

By applying OLS, the x -variable (y_m in the MSC and y_i in the ISC) is assumed to be free from any measurement error.²² This assumption is unlikely to hold for both scatter corrections, and disregarding measurement errors in either y_m or y_i may lead to attenuation of the slope estimate^{23,24} with possible consequences for the MSC or ISC corrected spectra. The potential influence of measurement errors associated with the set-mean or with the individual sample spectra needs to be recognised and an appropriate type II regression model, also called 'errors-in-variables' (EIV, errors-in-both-variables) or measurement error model,²³ used to estimate any of the slopes ($\hat{\beta}_{\text{MSC},i}$ or $\hat{\beta}_{\text{ISC},i}$) required for scatter correction. There is a range of type II regression models and the choice of the most appropriate in each case will depend on the ratio of measurement error variances of the y - and x -variables.^{25,26} These models have wider application when relationships between random variables are to be established.^{27,28} The objectives of this study are to examine the use of type II regression models (maximum likelihood and the specific cases of major axis [MA] and reduced major axis [RMA]) in the scatter correction of NIR spectral data, and to assess their impact on the slope estimate in MSC and on the development of calibration equations.

2 | TYPE II REGRESSION MODELS

The maximum likelihood (ML) estimate of the slope for the orthogonal regression ($\hat{\beta}_{\text{ML}}$) is calculated as

$$\hat{\beta}_{\text{ML}} = \frac{\hat{\sigma}_y^2 - \lambda_{\text{ML}} \hat{\sigma}_x^2 + \sqrt{(\hat{\sigma}_y^2 - \lambda_{\text{ML}} \hat{\sigma}_x^2)^2 + 4\lambda_{\text{ML}} \hat{\sigma}_{xy}^2}}{2\hat{\sigma}_{xy}} \quad (3)$$

where $\hat{\sigma}_x^2$ and $\hat{\sigma}_y^2$ are the sample variances of x and y , respectively, $\hat{\sigma}_{xy}$ is the sample covariance of x and y , and λ_{ML} is the ratio of the total error variances of y and x , that is

$$\lambda_{\text{ML}} = \hat{\sigma}_\epsilon^2 / \hat{\sigma}_\delta^2$$

where $\hat{\sigma}_\epsilon^2$ is the estimator of the error variance of a single y -value and $\hat{\sigma}_\delta^2$ is the estimator of the error variance of a single x -value, with the assumption that both $\hat{\sigma}_\epsilon^2$ and $\hat{\sigma}_\delta^2$ are constant over the range of the data.^{28,29} An alternative form of Equation 3 is Deming's formula^{30,31}

$$\hat{\beta}_{\text{ML}} = U + \sqrt{U^2 + \lambda_{\text{ML}}}$$

where

$$U = \frac{\hat{\sigma}_y^2 - \lambda_{\text{ML}} \hat{\sigma}_x^2}{2\hat{\sigma}_{xy}}$$

In the context of NIR analysis and MSC application, Equation 3 has MA and RMA as two special solutions.^{23,25,32} Assuming justifiably that $\hat{\sigma}_\epsilon^2$ and $\hat{\sigma}_\delta^2$ are equal (i.e., λ_{ML} equals 1), the MA regression model may be adopted, so that

$$\hat{\beta}_{\text{MA}} = \frac{\hat{\sigma}_y^2 - \hat{\sigma}_x^2 + \sqrt{(\hat{\sigma}_y^2 - \hat{\sigma}_x^2)^2 + 4\hat{\sigma}_{xy}^2}}{2\hat{\sigma}_{xy}}$$

where $\hat{\beta}_{\text{MA}}$ is the slope of the MA regression. Similarly, RMA (or 'y on x' and 'x on y' or geometric mean functional relationship) regression model may be appropriate when $\hat{\sigma}_\epsilon^2$ and $\hat{\sigma}_\delta^2$ are assumed to be proportional to $\hat{\sigma}_y^2$ and $\hat{\sigma}_x^2$ respectively, giving

$$\lambda_{\text{ML}} = \hat{\sigma}_y^2 / \hat{\sigma}_x^2$$

and thus from Equation 3, the slope for RMA regression ($\hat{\beta}_{\text{RMA}}$) is estimated as

$$\hat{\beta}_{\text{RMA}} = \text{sgn}(\hat{\sigma}_{xy}) \times \frac{\hat{\sigma}_y}{\hat{\sigma}_x}$$

If the data scales are the same for x and y then MA and RMA have the property, unlike OLS, that the slope of $y = f(x)$ is the reciprocal of the slope of $x = f(y)$. As a result, the RMA slope is the geometric mean of the 'y-on-x' and 'x-on-y' regression slopes

$$\hat{\beta}_{\text{RMA}} = \sqrt{\hat{\beta}_{y|x} / \hat{\beta}_{x|y}}$$

When applied to MSC and ISC procedures, this means that

$$\hat{\beta}_{\text{RMA},i} = \sqrt{\hat{\beta}_{\text{MSC},i} / \hat{\beta}_{\text{ISC},i}}$$

2.1 | Methodology for type II regression analysis

The method called functional relationship estimation by maximum likelihood (FREML) was applied to estimate the best fit linear relationships between two variables where both variables have significant uncertainties.³³ ML type II regression with separate λ_{ML} values (not constant) at each wavelength was fitted using the Excel FREML add-in developed by the Analytical Methods Committee (AMC) of the Royal Society of Chemistry (free download from https://www.rsc.org/globalassets/03-membership-community/connect-with-others/through-interests/divisions/analytical-division/amc/software/amc_freml_tcm18-26353.zip) from the algorithms described by Ripley and Thompson³⁴ and AMC.³³ For other type II regression methods (MA or RMA) the procedure RLFUNCTIONAL^{35,36} of the GENSTAT package³⁷ was used.

3 | IMPACT OF REGRESSION MODELS ON NIR SPECTRA

NIR operational procedures in the laboratory include scanning a set of samples to obtain the spectrum for each sample. It may be good laboratory practice to scan the same set of samples several times (on different days) to measure the variability imparted by the NIR spectrophotometer operating in a specific environment (such as ambient conditions, e.g., temperature) each day the samples are scanned. This does not include other components of variation such as particle size, particle orientation, path length, moisture, packing density, etc., which will influence the measurement errors. With a set of samples freshly packed and re-packed, a number of scans can be taken for each sample, so that the variation at each wavelength can be examined and a suitable estimate of λ_{ML} can be calculated from that error variance in NIR spectra. Then, the ML corrected slope ($\hat{\beta}_{\text{ML}}$) can be estimated. Assumptions about $\hat{\sigma}_\varepsilon^2$ and $\hat{\sigma}_\delta^2$ can be confirmed to test the suitability of the special cases of MA or RMA.

For this purpose, eight randomly selected grass samples were used (Data Set 1). Samples had been taken from eight different grazing plots sown with mixtures of three fodder grass varieties (namely DART, EPIC and MAGIC). The samples were collected at the third Spring cut, freeze-dried and ground (1 mm sieve). Each sample was scanned (FOSS NIRSystems 6500, FOSS UK Ltd., Warrington, UK) on three consecutive days. Each day, the samples were repacked to capture most of the factors that contribute to the measurement errors. Each time a given sample was scanned will be referred to as a run, with some inevitable variability among runs. The resultant spectra comprised two segments, specifically the 400–1,098 nm and 1,100–2,498 nm spectral ranges. From repeated scans of such a set of samples the reproducibility of NIR spectra was assessed. Sample mean spectra are illustrated in Figure 1.

Within each spectral range (either 400–1,100 nm or 1,100–2,498 nm), run-to-run standard deviation or variance ($\hat{\sigma}_{\text{run},j}^2$) at each wavelength of the j -th sample spectrum (one of the eight samples scanned, $j = 1 \dots 8$) was calculated. The

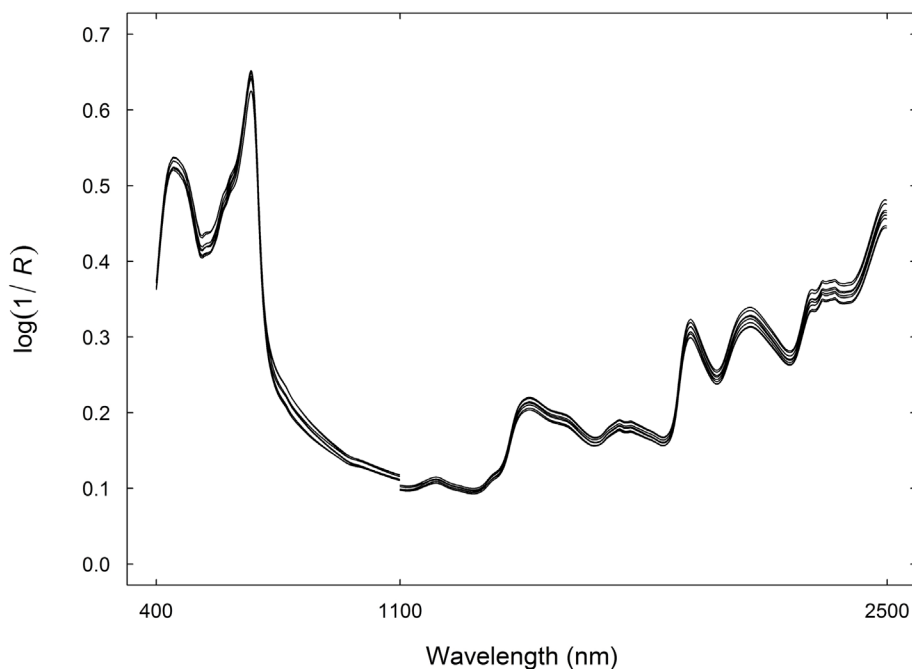


FIGURE 1 Mean near infrared spectra for eight dried and ground grass samples.

baseline of the NIR spectra is generally nonlinear. For example, the baseline across the 1,100–2,498 nm region showed an increasing and positively curving trend. The trends in standard deviation reflect the pattern in the baseline (Figure 2). For any NIR system it is desirable to know the size of measurement errors or uncertainty in the spectra of various types of substrates. The main reason for differential measurement errors is due to variable interactions between incident energy from the spectrophotometer and the attributes of the test material. If these interactions are substantial, then the measurement error variance for a set-mean spectrum may be different from the error variance of the individual samples even when the sample set substrates are homogeneous. Taking baseline shifts into account by expressing standard deviation relative to the sample mean (i.e., as coefficient of variation in %) gives a clearer indication of how variability changes across the wavelength range (Figure 3).

Approximate reproducibility ($\hat{\sigma}_R$)^{38,39} is generally taken as $1.5\hat{\sigma}_{\text{run},j}$ leading to an expanded uncertainty estimate of $2\sqrt{2}\hat{\sigma}_R$. Median expanded uncertainty values calculated for the eight sample spectra and expressed as a percentage of the respective wavelength-specific sample spectrum mean ranged from 3.4 to 15.5% for the 400–1,098 nm range and from 3.6 to 13.2% for the 1,100–2,498 nm range. Clearly the run-to-run variance component can be quite large and laboratory-to-laboratory variance is likely to be at least similar if not greater. As good practice, any calibration prediction model should include these components of variance.

Replacing the OLS regression coefficients in Equation 1 with estimates from type II regression, for example ML, gives the MSC corrected spectra as

$$y_{\text{MSC},i} = (y_i - \hat{\alpha}_{\text{ML},i}) / \hat{\beta}_{\text{ML},i}$$

In order to calculate these type II regression coefficients, the first step is to obtain a value for λ_{ML} .²⁸ On the one hand, run-to-run variance (which includes repacking process variance components) for the eight samples was calculated and assumed to represent the measurement errors for each individual spectrum. On the other hand, the run-to-run means and variances for the eight spectra were used to calculate the variance of the combined set mean ($\hat{\sigma}_{\text{run},m}^2$) using meta-analysis.^{40,41} With estimates of measurement errors for each of the eight mean spectra and their combined set mean, the ratio

$$\lambda_{\text{ML}} = \hat{\sigma}_{\text{run},j}^2 / \hat{\sigma}_{\text{run},m}^2$$

can be calculated (Figure 4).

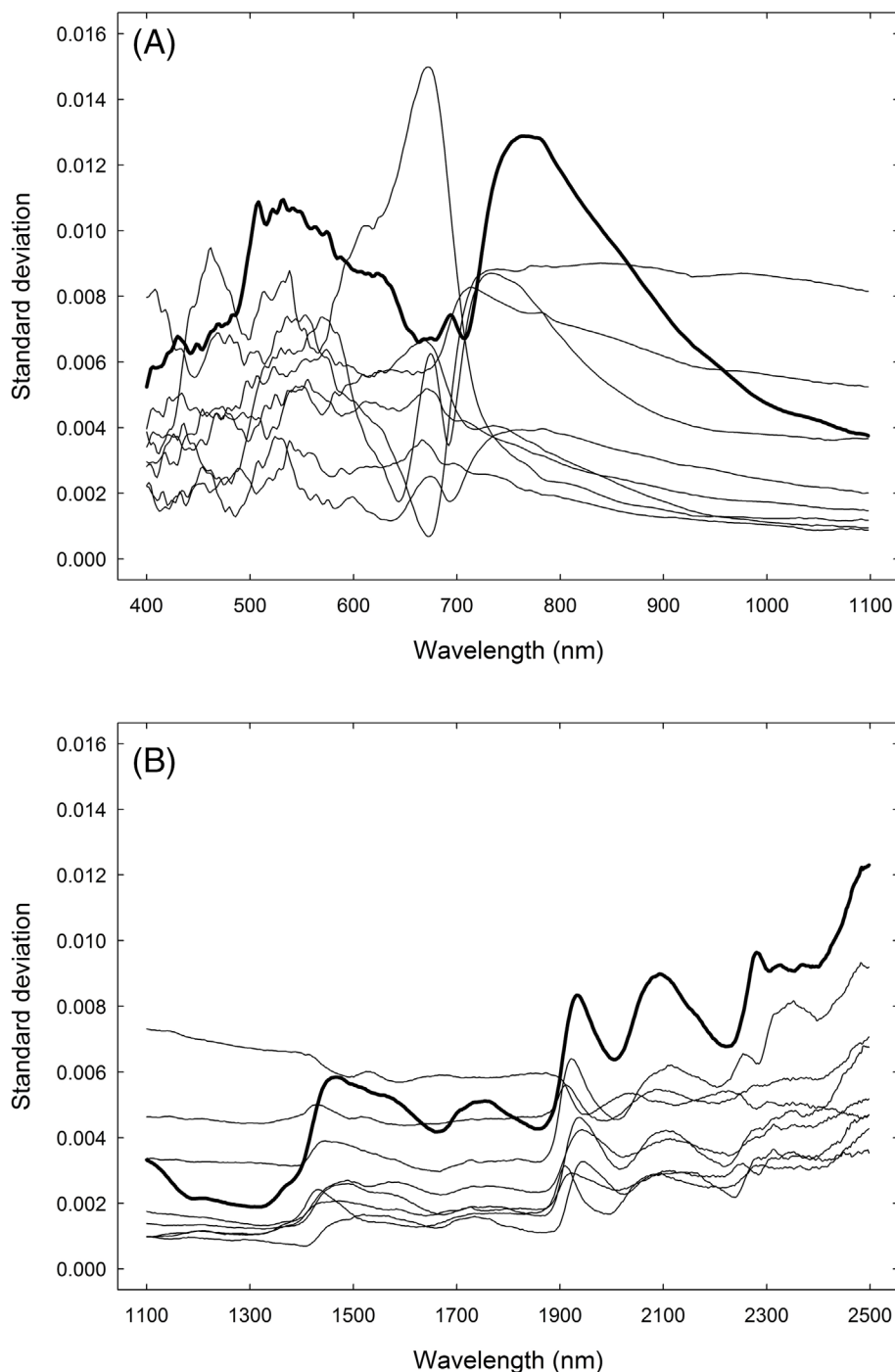


FIGURE 2 Wavelength-specific standard deviation associated with the eight mean sample spectra ($\hat{\sigma}_{\text{run},j}$) and the combined set mean spectrum ($\hat{\sigma}_{\text{run},m}$; bold) of Data Set 1 within the (A) 400-1,048 nm and (B) 1,100-2,498 nm regions.

With this limited data set, λ_{ML} tended to be positively skewed both within and across spectra. Median λ_{ML} values for individual samples ranged from 0.0516 to 0.8249 with median 0.1290 for the 400 - 1,098 nm region, and from 0.1092 to 1.2108 with median 0.2139 for the 1,100 - 2,498 nm region. ML was used to apply MSC correction to each of the eight sample mean spectra using the Excel FREML add-in.³³ Relative to OLS, slope bias was evident for ML calculated using the median λ_{ML} for each sample. With the ML solution slope estimates increased by 0.0048 to 0.0839% for the 400-1,098 nm range and by 0.0005 to 0.0228% for the 1,100-2,498 nm range. These differences may seem trivial but the rotational effect of the ML regression coefficients is enough to cause some degree of spectral distortion. The consequences of failing to take measurement errors in the x -variable into account by simply using OLS rather than ML to

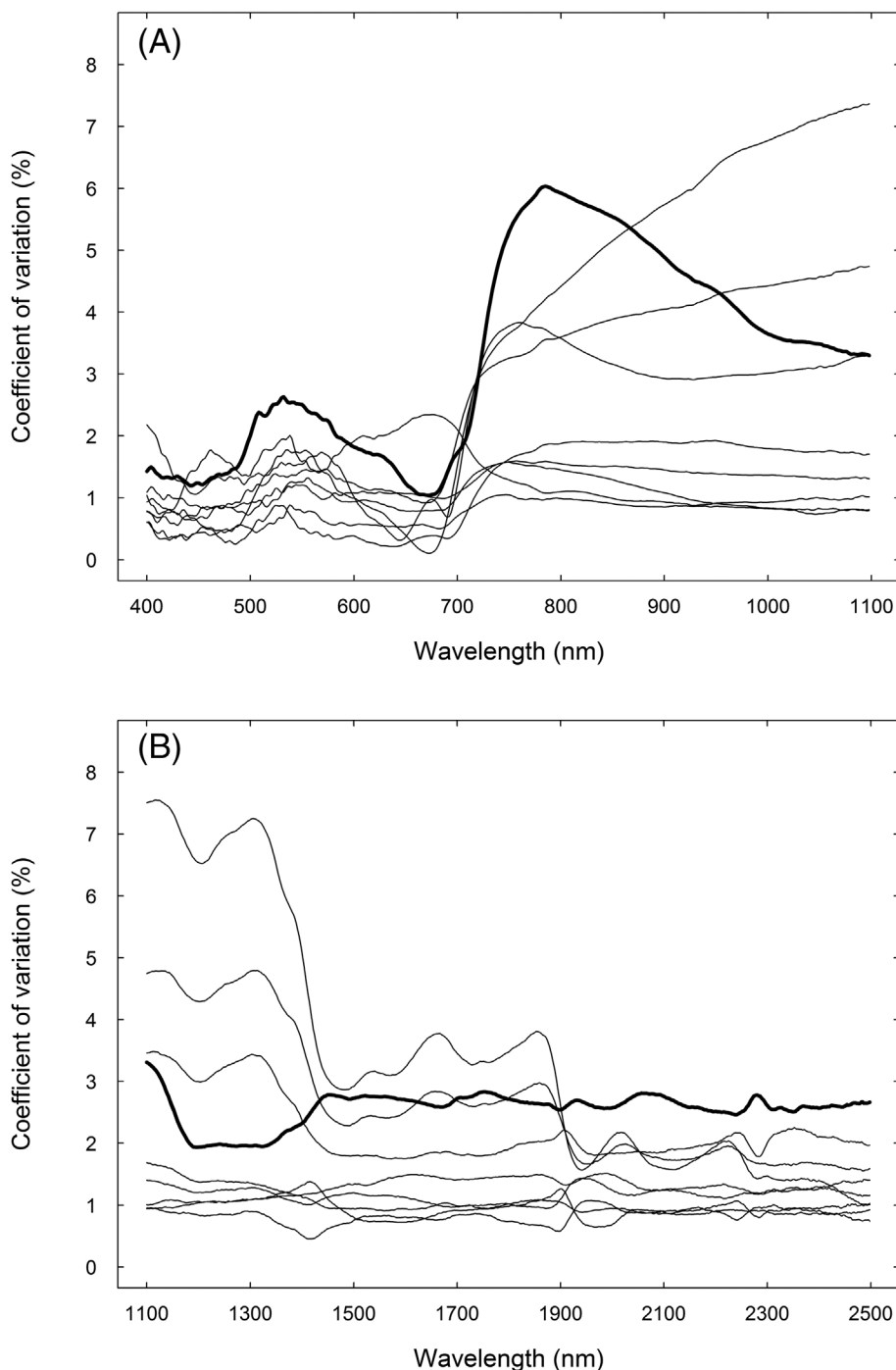


FIGURE 3 Wavelength specific coefficient of variation for the eight mean sample spectra and combined set mean spectrum (bold) of Data Set 1 across the (A) 400-1,098 nm and (B) 1,100-2,498 nm regions.

calculate MSC spectra are illustrated in Figure 5. The switch-point is due to slope attenuation effect on the fitted regression lines. When the slope estimates for MSC obtained by either OLS or type II regression methods were compared across the 24 spectra of Data Set 1 (using a paired t -test), it was observed that the difference between both methods was statistically significant ($p < 0.001$), although the magnitude of the slope attenuation accounted for 0.03%. Nevertheless, any slope attenuation bias will result in overestimated y-intercept and downward spectral rotation of the fitted line over the relevant wavelengths. Reduction of slope bias will reduce spectral rotational errors.

Ideally λ_{ML} should be constant or can be assumed constant over the wavelength range for either MA or ML to be applied. If the assumption of equal variance (i.e., λ_{ML} equals 1) required for application of MA is not met, the ML

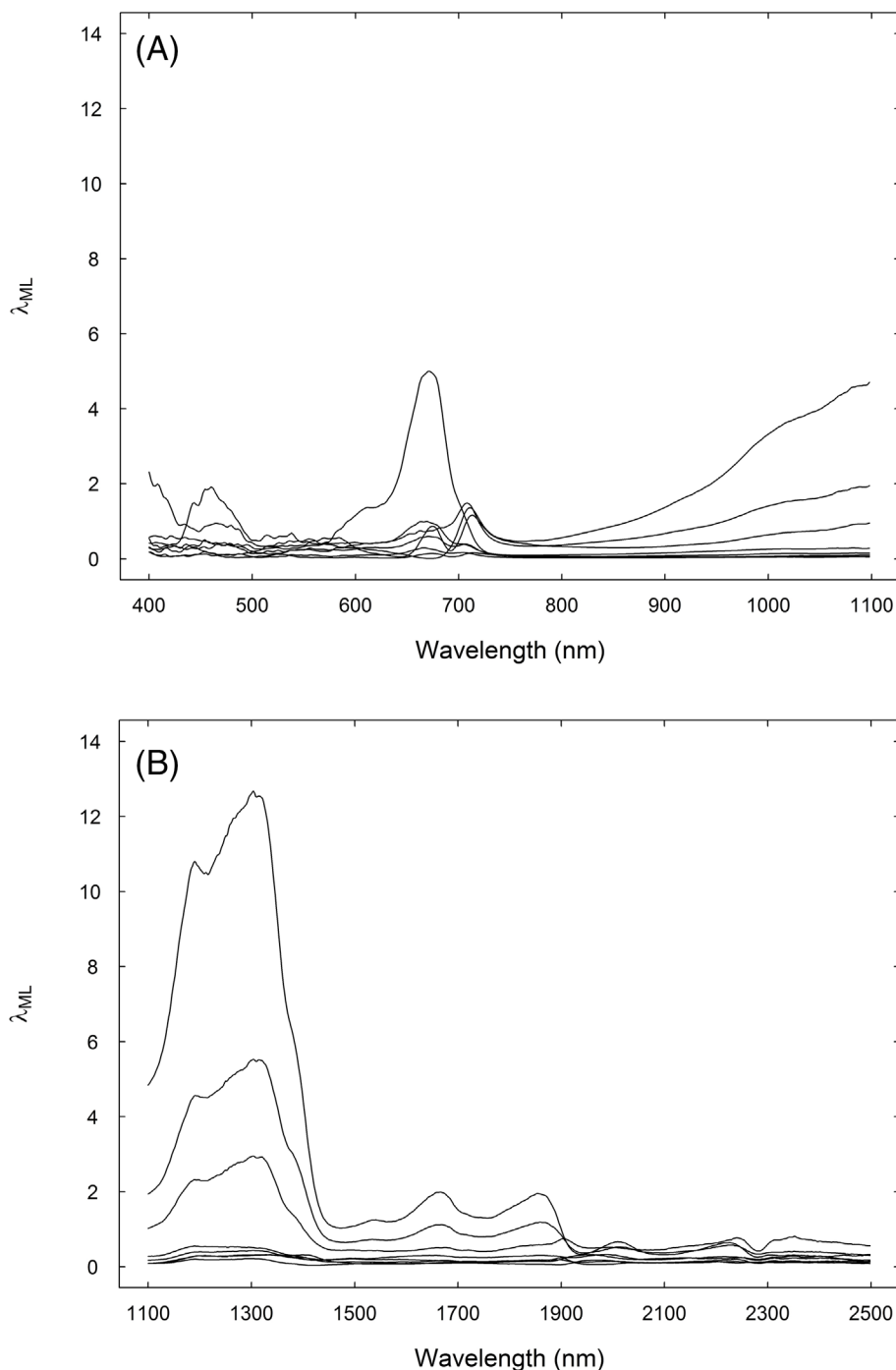


FIGURE 4 Wavelength specific λ_{ML} estimates for the eight mean spectra of Data Set 1 within the (A) 400-1,098 nm and (B) 1,100-2,498 nm regions.

solution has to be used. However, the assumption that λ_{ML} equals 1 may be reasonable in the context of NIR, especially when dealing with data from the same instrument in a single laboratory and each time a set of samples is scanned.

Within each single day (run), calculation of

$$\lambda_{ML} = \hat{\sigma}_{\epsilon}^2 / \hat{\sigma}_{\delta}^2$$

is rather difficult since an individual scan of a sample (on each day) do not provide estimates of measurement errors. The estimate of the variance of the set-mean spectrum on each day is among-sample-scan variance and not the

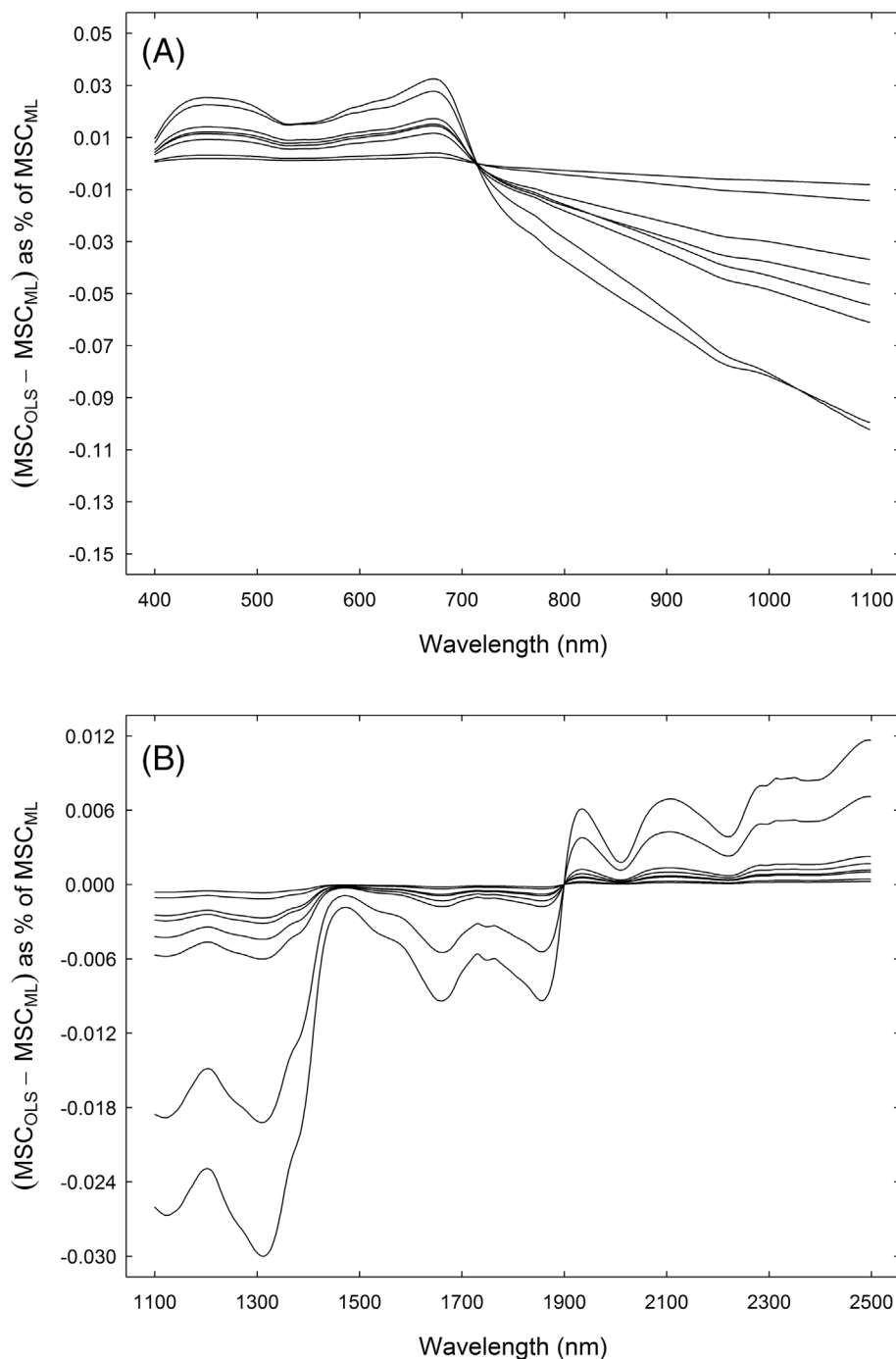


FIGURE 5 Spectral distortion associated with ordinary least squares slope bias relative to maximum likelihood regression using the median λ for each sample, (A) 400-1,098 nm and (B) 1,100-2,498 nm.

measurement variance. In the absence of measurement error variance, application of MSC within each day using type II regression to estimate the slope is only possible through MA with the assumption of equal measurement variances (i.e., λ_{ML} equals 1) or RMA with the assumption that the measurement variances are proportional to sample variances in y_i and y_m .

Depending on the relative size of the correlation, MSC with OLS will differ from MSC with MA, with RMA and with ML regression models. Spectral scans from homogeneous sample sets, such as Data Set 1, will give regression parameters quite similar for OLS, MA and ML regression, because the spectra are strongly correlated.

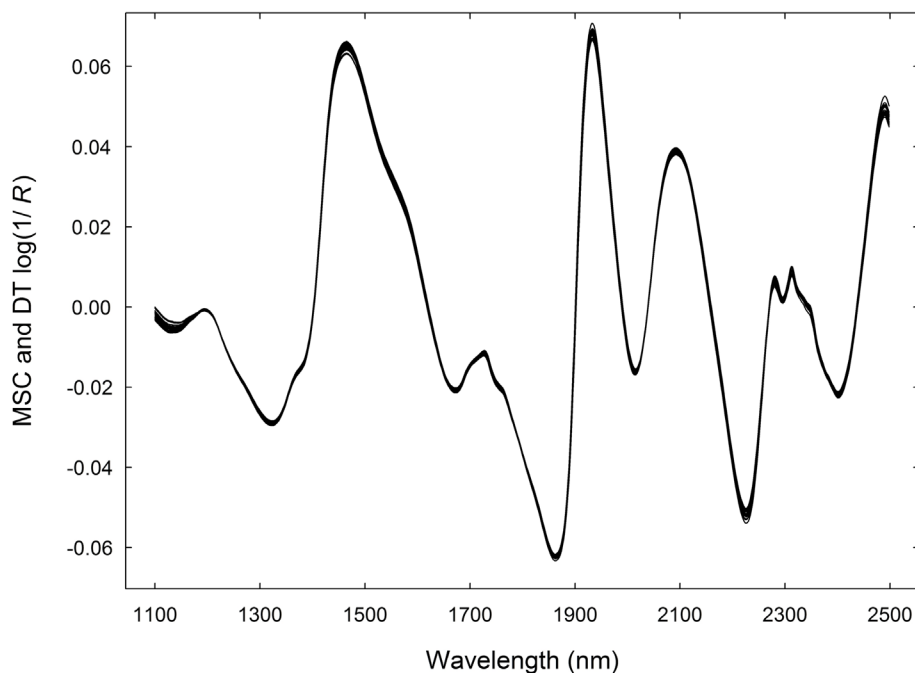


FIGURE 6 Spectra derived from Data Set 1 after multiplicative scatter correction (MSC) and de-trending (DT).

In addition to incorporation of type II regression into the MSC transformation, further pre-treatments should be considered. In the NIR calibration the common offset (\bar{y}_m) simply shifts the MSC spectra baseline from zero to the average of the set-mean spectrum level and therefore imparts enhanced set-mean dependency in each of the MSC spectra. Omitting the common offset such that

$$y_{\text{MSC},i} = (y_i - \bar{y}_i) / \hat{\beta}_{\text{ML},i}$$

will reduce the set-mean dependency to that acquired through the set-mean standard deviation, which is implicit to the estimate of slope considering that

$$\hat{\beta}_i = r_{i,m}(\hat{\sigma}_i / \hat{\sigma}_m)$$

Another option is to retain the regression-smoothed individual sample spectrum. By applying Equation (1), the individual sample spectrum is smoothed and certain spectral features are lost. Following this step an estimate of the set-mean spectrum is calculated using the smoothed individual sample spectra (Equation 2). Finally, MSC-spectra can be detrended by removing the curvilinear baseline using the quadratic function^{10,42} to highlight the remaining spectral features (Figure 6). More recently a spectral ratio method has been proposed for scattering correction.⁷

4 | EXTENT OF SLOPE ATTENUATION

The extent of slope attenuation can be illustrated more clearly with the less homogeneous Data Set 2, which comprised NIR spectra over the region of 1,100–2,498 nm from four coarse samples (average particle size 400 μm ; samples 1–4) and two finely powdered samples (average particle size 50 μm ; samples 5 and 6) of crystalline sucrose scanned on a PSCO Model 6250 monochromator.¹⁴ The divergence of the spectra associated with the sample form and particle size were evident. Thus, the powdered and more densely packed form showed decreased absorbances compared with the crystalline sucrose. MSC was applied to the individual sucrose spectra taking the set-mean spectrum as the reference. The

TABLE 1 Correlations ($r_{i,m}$) of individual sucrose spectra (Data Set 2) with the set-mean spectrum and regression parameter estimates (intercept $\hat{\alpha}$ and slope $\hat{\beta}$) for multiplicative scatter correction (MSC) pre-treatment under ordinary least squares (OLS) and major axis (MA) models.

Sample	$r_{i,m}$	OLS		MA	
		$\hat{\alpha}_{OLS,i}$	$\hat{\beta}_{OLS,i}$	$\hat{\alpha}_{MA,i}$	$\hat{\beta}_{MA,i}$
1	0.9992	0.0199	1.3122	0.0190	1.3135
2	0.9998	0.0126	1.2585	0.0124	1.2588
3	0.9998	-0.0220	1.2170	-0.0222	1.2173
4	0.9990	0.0323	1.2681	0.0313	1.2696
5	0.9922	-0.0260	0.4407	-0.0268	0.4418
6	0.9937	-0.0168	0.5036	-0.0176	0.5049

regression parameters for MSC pre-treatment were estimated by using either OLS or MA regression models. Linear regression by OLS was fitted using the GENSTAT package³⁷ whereas the GENSTAT procedure RLFUNCTIONAL^{35,36} was used for MA regression. Without repeated scans, λ_{ML} could not be estimated for this data set. Thus, to correct the OLS slope estimates for the application of MSC, MA estimates had to be used as the spectral scales are identical for both y- and x-variables. Relative increases in slope estimates from MA compared with OLS, i.e., $[\hat{\beta}_{MA,i} - \hat{\beta}_{OLS,i}] / \hat{\beta}_{OLS,i}$, ranged from 0.020 to 0.257% (Table 1) with differences in regression parameters dependent upon the size of correlation. Powdered samples (5 and 6) showed to be slightly less correlated with the set mean than crystalline samples. For these two spectra there was a greater change in regression parameter estimates. As a result, the correlation coefficient between the relative increase in slope and $r_{i,m}$ for the values in Data Set 2 was -0.949. Any substrate sample set will have a range of correlations which in turn will affect the OLS regression parameters for the application of MSC correction and create some distortions among the MSC-corrected spectra, as illustrated above. The MA and RMA type II regression models are useful only when $r_{x,y}$ is significant, which is generally true for NIR spectra.

Differences between OLS and type II (errors-in-both-variables) regression methods in the slope estimate were also examined using simulated data with greater errors than in the observed spectra already evaluated. Three spectra from Data Set 3 (Lucerne herbage samples, see full description below) were used. In a first approach some bias was added to each spectrum over all the wavelengths (from 400 to 2,500 nm). The extent of the bias was random (within the range recorded across the 54 samples included in the data set) and it was either positive or negative, resulting in three simulated spectra over and over three under each observed NIR spectrum, for a total of 18 simulated spectra. The mean-spectrum across these simulated spectra was used as the reference to simulate a MSC by regressing each individual spectrum against the mean-spectrum. The slope of each linear regression was estimated by OLS or type II (MA or RMA) methods. MA and RMA resulted in similar slope estimates (average $\hat{\beta}_{MA}$ was 1.0034 (range 0.8766-1.1776) and average $\hat{\beta}_{RMA}$ 1.0033 (range 0.8775-1.1765)). However, both type II methods provided slope estimates significantly greater ($p < 0.05$) than that obtained with OLS (average $\hat{\beta}_{OLS}$ was 1.0000 [range 0.8710-1.1700]). This trend was observed for all the simulated spectra, showing that slope estimates were, on average, underestimated by 0.33% with OLS compared with type II regression methods. To explore if this underestimation could be enlarged if spectral noise was amplified, a second set of simulated spectra was derived by adding bias (randomly in magnitude and sign -positive or negative-) to each value (wavelength) of each spectrum. This resulted in simulated spectra showing a similar trend to the parent (observed) spectrum from which they were derived but with a ragged or saw-toothed profile around that trend. Five simulated spectra were obtained from each of the three original (real) spectra. Again, MA and RMA resulted in similar slope estimates (average $\hat{\beta}_{MA}$ was 1.0573 (range 0.9294-1.1687) and average $\hat{\beta}_{RMA}$ 1.0546 (range 0.9336-1.1598)). It was confirmed that slope estimates with both type II methods were significantly greater ($p < 0.001$) than those obtained with OLS (average $\hat{\beta}_{OLS}$ was 1.0009 [range 0.8768-1.1023]), but in this case the difference accounted for 5.63% (increase of $\hat{\beta}_{MA}$ vs. $\hat{\beta}_{OLS}$) and 5.36% (increase of $\hat{\beta}_{RMA}$ vs. $\hat{\beta}_{OLS}$). This analysis shows that OLS can result in a substantial slope attenuation (compared with type II methods) that is amplified as the spectral noise is increased. Note that the pair-wise correlations between individual and mean simulated smoothed spectra ranged from 0.9926 and 0.9999, whereas for the set with simulated ragged spectra, the range was between 0.9354 and 0.9559.

5 | IMPACT OF REGRESSION MODELS ON CALIBRATION AND VALIDATION

For assessing the application of scattering correction pre-treatments to NIR spectra, spectral and reference (chemical composition) data of animal feeds were used, a field where NIR technology has been extensively applied.^{43–46} To evaluate the impact of using type II regression models when applying MSC to spectral data on the development of calibration equations, NIR spectra from 54 Lucerne (cv. Timbale) herbage samples were used (Data Set 3) from a study to investigate the effects of cultivation date and method on the establishment of Lucerne in the UK. Thus, the samples were harvested from 1st and 2nd cuts of 27 plots sown either by conventional re-seeding or by direct drilling and either with or without a pre-cultivation herbicide. The 54 samples were freeze-dried and ground (1-mm screen). Chemical composition of each sample was determined by standard analytical methods. Total nitrogen (N) and water-soluble carbohydrates (WSC) were determined as described by Merry et al.,⁴⁷ whereas the analysis of neutral detergent fibre (NDF) was as described by Van Soest et al.⁴⁸ Chemical composition of the Lucerne herbage samples comprising Data Set 3 is summarised in Table 2. Aside, the samples were scanned on a NIRSystems Model 6500 spectrophotometer in the wavelength region between 1,100–2,498 nm at 2 nm intervals. Scanning was repeated in three separate runs (each sample was re-packed and scanned on three consecutive days) to provide an estimate of variance at each wavelength. Calibrations were derived to estimate chemical composition (total N, WSC and NDF on dry matter basis) from NIR spectra. Prior to calibration, the spectra were corrected by applying one of the pre-treatments described above. Individual spectrum scale equalising correction was used to derive SNV and detrended NIR spectra. These were considered as the reference corrected spectra free from regression model induced effects. Additionally, spectra were corrected by either original MSC or ISC, using OLS^{36,37} linear regression. Finally, spectra were corrected by MSC but fitting the type II ML regression method with separate λ_{ML} values using the Excel FREML add-in.³³ The mPLS algorithm in dedicated WINISI, Version 4.6.8 (Infrasoft International, State College, PA) was used for the NIR calibrations by multiple linear regression methods and internal cross validation with all 54 samples. Mathematical treatments implemented in the software and used for the calibration were: order of derivative function = 2, gap (length in nm) in data points over which the derivative is calculated = 6, number of data points (segment length) used in first smoothing = 4, and number of data points in the second smoothing = 1. In principle, all the 54 samples were used for calibration although those identified as outliers were excluded. The prediction performance in each case was assessed using calibration quality indices provided by the WINISI software: standard error of calibration (SEC), proportion of variance accounted for by the model (R^2), standard error of cross validation (SECV) and coefficient of determination in cross validation (denoted by the expression “1–VR”).

A subsequent calibration was performed by splitting the whole set (54 samples) into a calibration set of 40 samples and a validation set of 14 samples, to assess the influence of MSC (using type II regression) on external validation. As shown in Table 2, compositional data of the calibration set after splitting (40 samples) were similar to those of the whole set (54 samples). Calibration was obtained in this case using principal component regression (PCR) analysis

TABLE 2 Chemical composition (% on dry matter basis) from Data Set 3 (Lucerne herbage samples).

	Mean	Standard deviation	Median	Min	Max
Nitrogen					
Whole set ^a	2.965	0.6827	3.14	1.43	3.98
Calibration set ^b	2.895	0.7223	3.13	1.43	3.98
Water soluble carbohydrates					
Whole set ^a	12.08	6.221	9.79	6.08	29.42
Calibration set ^b	13.08	6.748	9.94	6.75	29.42
Neutral detergent fibre					
Whole set ^a	40.98	4.052	40.69	34.63	51.25
Calibration set ^b	41.57	4.272	41.69	34.63	51.25

^aWhole set = 54 samples of Lucerne herbage;

^bCalibration set after splitting = 40 samples of Lucerne herbage.

(GENSTAT RIDGE procedure⁴⁹) to prevent or reduce multicollinearity, a dominant feature of NIR spectral wavelengths that affects calibration modelling by multiple regression. Also, calibration was performed using partial least squares (PLS) regression (GENSTAT PLS procedure⁵⁰). Calibrations using PCR or PLS were similar, with high concordance coefficients⁵¹ between both methods (0.988, 0.977 and 0.969 for N, WSC and NDF data, respectively). However, as the reproducibility of measured (reference) data was slightly improved with PCR compared with PLS regression (concordance coefficients -PCR vs. PLS- of 0.994 vs. 0.981 for total N; 0.959 vs. 0.933 for WSC and 0.929 vs. 0.889 for NDF), PCR calibrations were retained for further analyses. The relationship between NIR and reference method values was used to assess calibration (SEC, R^2 and SECV using the calibration set) and validation (R^2 and standard error of prediction [SEP] with the 14 samples of the validation set). Additionally, the prediction performance was evaluated on the validation set using statistics⁵² such as mean square prediction error (MSPE) and its partition into bias, slope and random components,⁵³ Lin's concordance correlation (CC) coefficient^{51,53} and modelling efficiency (ME). This latter statistic was calculated as⁵⁴

$$ME = 1 - \frac{\sum (ref_i - nir_i)^2}{\sum (ref_i - avg)^2}$$

where ref_i are the reference method values, nir_i the values estimated from NIR spectra and avg the average for the validation set.

Calibration performance observed with the whole Data Set 3 (54 samples) is summarised in Table 3. The number of terms in the calibration equations was six for N and WSC and five for NDF, and the number of identified and excluded outliers was three for N and only one for WSC and NDF. In all cases, NIR spectra showed a remarkable potential to predict chemical composition of Lucerne estimating mean, minimum and maximum N, WSC and NDF similar to the

TABLE 3 Comparison of calibration statistics (whole Data Set 3: 54 samples) derived from standard normal variate and de-trended near infrared spectra and those derived from spectra after modified multiplicative scatter correction by three different approaches.

Item ^a	Pre-treatment ^b	Mean	Min	Max	SEC ^c	R^2	SECV ^c	1-VR
N	Original spectra	2.98	1.43	3.98	0.067	0.990	0.095	0.980
	SNV & detrend	2.98	1.43	3.84	0.044	0.996	0.072	0.988
	MSC-OLS	2.98	1.43	3.84	0.044	0.996	0.072	0.988
	ISC-OLS	2.98	1.43	3.84	0.043	0.996	0.072	0.988
	MSC-ML	2.98	1.43	3.84	0.043	0.996	0.072	0.988
WSC	Original spectra	11.6	6.08	27.1	0.428	0.994	0.774	0.981
	SNV & detrend	11.9	6.08	29.4	0.422	0.995	0.705	0.987
	MSC-OLS	11.9	6.08	29.4	0.421	0.995	0.705	0.987
	ISC-OLS	11.9	6.08	29.4	0.423	0.995	0.704	0.987
	MSC-ML	11.9	6.08	29.4	0.424	0.995	0.705	0.986
NDF	Original spectra	41.0	34.6	51.2	1.597	0.845	1.734	0.813
	SNV & detrend	40.9	34.6	51.2	1.186	0.916	1.580	0.847
	MSC-OLS	40.9	34.6	51.2	1.188	0.915	1.585	0.846
	ISC-OLS	40.9	34.6	51.2	1.187	0.915	1.586	0.846
	MSC-ML	40.9	34.6	51.2	1.189	0.915	1.586	0.846

^aItem (all in % of dry matter): N = nitrogen; WSC = water soluble carbohydrates; NDF = neutral detergent fibre.

^bSpectral pre-treatment: SNV & detrend = standard normal variate and detrending; MSC-OLS = multiplicative scatter correction with ordinary least squares regression; ISC-OLS = inverted signal correction with ordinary least squares regression; MSC-ML = multiplicative scatter correction with maximum likelihood (Deming) regression (non-constant λ_{ML}).

^cGoodness of fit: SEC = standard error of calibration; R^2 = proportion of variance accounted for by the model; SECV = standard error of cross validation; 1-VR = coefficient of determination in cross validation.

reference values (Table 2), with relatively small errors of calibration and cross-validation and high coefficients of determination. Calibration performance was improved when any pre-treatment was applied, as calibration errors were reduced, and coefficients of determination increased. However, there were no differences among the different pre-treatments in the calibration performance, all of them resulting in almost identical predicted values and statistics of goodness-of-fit.

Using external validation (splitting the whole Data Set 3 into a calibration and a validation sub-set) revealed some differences among pre-treatments (Tables 4 and 5). In this case, calibration performance was slightly improved by pre-treatments with N and NDF data, but not with WSC data. ISC correction did not affect the calibration observed with the original spectra. Calibration for N data was improved with SNV-detrend and MSC pre-treatments, for NDF only with MSC. Applying a type II regression model (MSC-ML) reduced SECV (cross validation) for N and NDF compared with the same correction (MSC) using OLS. Goodness-of-tatistics (Table 5) and observed (reference) vs. predicted (NIR estimates) plots (Figure 7) showed a good predictive performance of NIR spectra to estimate N and WSC content in Lucerne. In the case of N, the goodness-of-prediction was improved with SNV-detrend and MSC pre-treatments (reduced SEP and increased R^2), but this improvement was not observed for WSC. As for NDF, validation showed a poor predictive performance. These results were confirmed when MSPE and concordance analyses were applied to observed and predicted values in the validation subset for the comparison of models. For NDF, concordance between observed and predicted values was not high (< 0.5 , Figure 7), but it was improved applying SNV-detrend or MSC pre-treatments compared with the use of original spectra (Table 5). This can be attributed to a reduced overall bias considering the lesser percentage of this component in total MSPE. In the case of WSC, a substantial deviation from isopleth of the slope relating observed with predicted values was detected, and scatter correction did not improve concordance or reduce MSPE. The prediction of N from NIR spectra was noteworthy, with high concordance ($CC > 0.925$) and small MSPE, overall bias and deviation of slope from isopleth. The prediction of N content was slightly improved with SNV-detrend and MSC pre-treatments compared with that

TABLE 4 Comparison of calibration and validation statistics (split Data Set 3) derived from standard normal variate and de-trended near infrared spectra and those derived from spectra after modified multiplicative scatter correction by three different approaches.

Item ^a	Pre-treatment ^b	Calibration (n = 40) ^c			Validation (n = 14) ^c				
		SEC	R ²	SECV	Mean	Min	Max	SEP	R ²
N	Original spectra	0.210	0.956	0.459	3.28	2.10	3.78	0.154	0.885
	SNV & detrend	0.181	0.967	0.403	3.27	2.14	3.81	0.136	0.906
	MSC-OLS	0.180	0.968	0.406	3.26	2.13	3.82	0.139	0.907
	ISC-OLS	0.210	0.956	0.459	3.28	2.15	3.83	0.154	0.885
	MSC-ML	0.182	0.967	0.401	3.26	2.14	3.79	0.141	0.904
WSC	Original spectra	0.378	0.857	3.59	9.14	4.51	18.24	0.772	0.941
	SNV & detrend	0.378	0.857	3.46	9.45	3.38	18.51	1.177	0.863
	MSC-OLS	0.379	0.856	3.39	9.54	3.73	18.43	1.151	0.869
	ISC-OLS	0.378	0.857	3.59	9.03	4.40	18.13	0.772	0.941
	MSC-ML	0.378	0.857	3.55	9.51	3.20	18.64	1.154	0.869
NDF	Original spectra	0.479	0.771	2.42	40.1	37.2	45.3	2.66	0.182
	SNV & detrend	0.478	0.772	2.49	39.7	37.2	44.4	2.62	0.207
	MSC-OLS	0.468	0.781	2.60	39.6	36.9	44.5	2.65	0.191
	ISC-OLS	0.479	0.771	2.42	40.1	37.2	45.3	2.66	0.182
	MSC-ML	0.486	0.764	2.30	39.6	37.3	44.3	2.58	0.234

^aItem (all in % of dry matter): N = nitrogen; WSC = water soluble carbohydrates; NDF = neutral detergent fibre.

^bSpectral pre-treatment: SNV & detrend = standard normal variate and detrending; MSC-OLS = multiplicative scatter correction with ordinary least squares regression; ISC-OLS = inverted signal correction with ordinary least squares regression; MSC-ML = multiplicative scatter correction with maximum likelihood (Deming) regression (non-constant λ_{ML}).

^cGoodness of fit: SEC = standard error of calibration; R² = proportion of variance accounted for by the model; SECV = standard error of cross validation; SEP = standard error of prediction.

TABLE 5 Comparison of prediction performance (validation sub-set from Data Set 3: 14 samples) derived from standard normal variate and de-trended near infrared spectra and those derived from spectra after modified multiplicative scatter correction by three different approaches.

Item ^a	Pre-treatment ^b	MSPE ^c	Bias	Slope	Random	CC ^c	ME ^c
N	Original spectra	4.63	7.46	1.44	91.1	0.930	0.874
	SNV & detrend	4.16	6.11	1.69	92.2	0.945	0.898
	MSC-OLS	4.09	4.64	0.76	94.6	0.930	0.901
	ISC-OLS	4.68	9.58	1.41	89.0	0.929	0.871
	MSC-ML	4.17	4.35	2.36	93.3	0.944	0.897
WSC	Original spectra	10.8	0.89	47.4	51.7	0.953	0.886
	SNV & detrend	17.8	2.31	53.7	44.0	0.889	0.689
	MSC-OLS	16.8	3.93	48.7	47.4	0.898	0.724
	ISC-OLS	10.9	3.94	45.8	50.3	0.952	0.883
	MSC-ML	18.0	2.85	56.0	41.1	0.888	0.681
NDF	Original spectra	6.82	9.60	5.83	84.6	0.370	0.033
	SNV & detrend	6.37	2.28	3.59	94.1	0.404	0.157
	MSC-OLS	6.47	1.42	5.34	93.2	0.396	0.132
	ISC-OLS	6.85	10.33	5.78	83.9	0.367	0.025
	MSC-ML	6.19	1.69	2.10	96.2	0.427	0.203

^aItem (all in % of dry matter): N = nitrogen; WSC = water soluble carbohydrates; NDF = neutral detergent fibre.

^bSpectral pre-treatment: SNV & detrend = standard normal variate and detrending; MSC-OLS = multiplicative scatter correction with ordinary least squares regression; ISC-OLS = inverted signal correction with ordinary least squares regression; MSC-ML = multiplicative scatter correction with maximum likelihood (Deming) regression (non-constant λ_{ML}).

^cGoodness of fit: MSPE = mean square prediction error, with partition into bias, slope and random (as % of the MSPE); CC = Lin's concordance correlation coefficient; ME = model efficiency.

observed with the original spectra. Concordance was improved when ML was used to apply MSC compared with OLS. Our results show that there is not a unique solution valid for all situations. The chemical fraction under study had a critical influence on the outcome, with more consistent results when a more defined chemical component (N) was examined, in comparison with less homogeneous fractions (WSC or NDF). Greater sample sizes of the calibration and validation sets would be required to examine the effects of scatter correction approaches on the prediction of these fractions. The use of spectral scatter correction pre-treatments has been evaluated with subtle and variable results depending on the data set used and the variable under assessment.^{21,42,55,56} MSC is applied on a set of several spectra for a given sample (with unique chemical composition) obtained to account for the different sources of variability (within sample) that can affect the NIR spectroscopy. The assumed distribution of residuals ϵ (reasonably expected to be independent) and the trade-offs between model shortcomings and utility are generally favourable in the case of NIR spectroscopy, and make MSC an effective tool for the correction of multiplicative offset noise. Garrido-Varo et al.⁴² concluded that SNV and detrending of NIR spectra contributed to better discrimination between agrifood products. Fernández-Cabanas et al.⁵⁶ observed that spectra pre-treatments allow for detecting soil contamination in vegetal samples. Helland et al.²¹ reported a multiple comparison of several pre-treatments and observed that they were all closely related, with subtle differences among them, so that in practice the use of one or another did not have a relevant implication. Jiao et al.⁵⁷ concluded that there is not much to be gained in using scattering correction, and the size of such improvement would be determined by the sample heterogeneity. Scattering correction should be part of an ensemble pre-processing strategy to select the combination of baseline and scattering corrections, smoothing and scaling procedures that prove most suitable in each case.^{58,59} In this context, it seems well worth considering the use of type II regression models for assessing the application of MSC aiming at improving the goodness of prediction from NIR spectra.

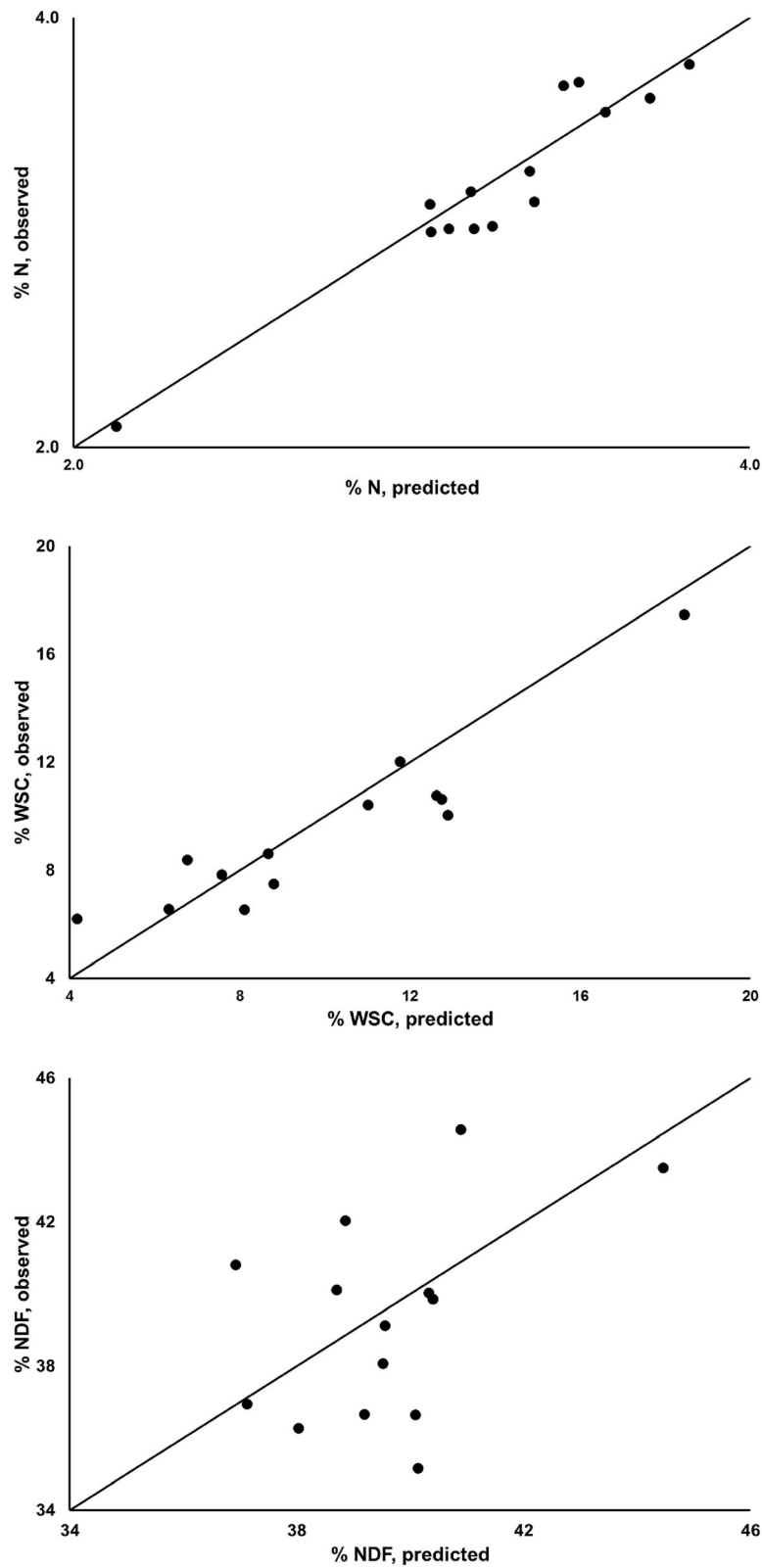


FIGURE 7 Observed vs. predicted scatter plots (for the validation set) resulting from the prediction of chemical composition (N = nitrogen; WSC = water soluble carbohydrates; NDF = neutral detergent fibre) from near-infrared spectroscopy.

6 | CONCLUDING REMARKS

It is good practice to identify appropriate methodology and most of the sources of measurement errors associated with any data. Repeatability, reproducibility, and other variance components are required to estimate expanded uncertainty associated with results from analytical laboratories. Regression models that account for errors in both y - and x -variables might have to be considered with respect to the application of MSC transformation, because this correction involves relationships between random variables. Regression in the case of simple MSC is necessarily linear. For multi-linear and nonlinear situations parallel methodologies may be found.

Knowledge of the various variance components is opportune in identifying appropriate regression models. Best practice will be to analyse samples from a validation set with replication, preferably scanned several times (different days or after repacking) to capture reproducibility variation. Relatively high correlation between the set-mean spectrum and the individual spectra ensures relatively small attenuation in the estimate of OLS slope. The MA approach can be a useful choice to correct slope estimates (and hence estimates of the intercept) for MSC application, particularly in the absence of repeated scans. The correction may have implications for calibration modelling and can also be relevant when predictions from a validation set are to be compared with their corresponding laboratory or reference values. The use of scattering correction and type II regression models for MSC might have limited impact on the goodness of prediction from NIR spectra, but their use case by case is warranted to test what extent prediction of reference values can be improved. These methods can be easily included as an extra option in dedicated NIR software.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from Dr. Sue Lister.

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