HOW OFTEN DO REFERENCES NEED TO BE MEASURED WHEN USING A NEAR INFRARED DIODE ARRAY SPECTROMETER

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ABSTRACT

The stability of a diode array spectrometer in the near infrared region has been investigated in two experiments where the frequency of passing of dark and white references, which need manual intervention, was varied. In the first experiment an initial pair of references was used to standardise spectra taken over the next four days. In the second, references were taken at hourly intervals over a period of ten hours. The conclusion is that in a reasonably well controlled environment the spectrometer is stable over long periods, and the passing of hourly references conveys no advantage. An important implication is that this spectrometer may be used in on-line applications without the need to construct an automatic mechanism to measure references.

Keywords: NIRS technology, diode array spectrometer, near infrared spectra, instrument references, instrument calibration.

Abbreviations and/or acronyms

h: hours;

NIRS: near infrared reflectance spectroscopy;

nm: nanometres;

ppm: parts per million;

R: reflectance;

RMS: root mean square (deviation);

t: time

VISNIR: visible and near infrared (instrument);

1) INTRODUCTION

As is well-known, near-infrared spectroscopy (NIRS) is a non-destructive technique that has been employed in diverse areas such as chemical, petrochemical, pharmaceutical and textiles industries, biomedical research, and above all, for many different applications in the agro-food sector.[1,2] Worldwide, the animal feed industry has been one of the most benefited by the advances in NIRS technology. Today, large and small feed companies utilize NIRS in their laboratories for the analysis of incoming materials and finished product.[3]

In any given application, the selection of an appropriate spectrometer is conditioned by factors like wavelength range, resolution and accuracy, instrumental noise, speed of spectral acquisition, operating environment, lifetime, warranty and cost, etc. One further consideration that can become important, especially for on-line applications, is the system used by the instrument to measure the dark and white references needed to standardise the spectra. While some types of spectrometer have an internal and automatic mechanism for taking references, other types require regular manual intervention for this purpose. In an on-line application, in which it is usual to take thousands of spectra per day, it is not practical to pass dark and white references by hand with any great frequency. The question that arises is whether the instrument is sufficiently stable to perform well with long intervals between references.

For “at line” applications, in which the number of spectra measured is typically lower, it is possible to take the dark and white references at regular intervals. But at this

point, one can ask how often the references must be controlled to maintain stable results: every half an hour, every hour, every two hours?. The answer to this question is not easy. One can, for example, find in the literature for a given diode array spectrometer type these different recommendations: 30 minutes,[4] an hour,[5] every ten measures,[6] every measure.[7]

In this paper, the stability of a diode array spectrometer, in which reference measurements have to be made by hand, is investigated as a typical example of the instrumentation concerned. Two experiments applying different regimes for the taking of references were run. In the light of the results, some recommendations are proposed that could be useful for others who work with similar spectrometers.

2) MATERIAL AND METHODS

The tests were made using a VISNIR 45 Corona® (Carl Zeiss, Jena, Germany), visible and near-infrared spectrometer. The main features of this instrument are summarized in Table 1, according to the technical specifications supplied by the manufacturer.[8] It was not the intention of the authors either to advertise or criticise this particular instrument or manufacturer, but to regard the instrument as representative of a particular type.

The spectrometer was installed on a stationary module made by J. Haldrup a/s of Løgstør, Denmark, which allows the spectral measurement of a fixed point. Figures 1a and 1b show the appearance of this mechanism, where the spectrometer is directly

supported by a gyratory platform which is placed above a conveyor belt. For the test, the conveyor belt was used in static mode and a prismatic watertight cell (designed to avoid sample humidity changes) was placed on it. The prismatic cell contained approximately 50 grams of compound feed.

2.1) FIRST TEST SERIES

As was indicated at first, two types of tests were carried out. The methodology of the first type is described as follows: the spectrometer and the compound feed cell were placed in the laboratory 24 hours before starting the experiments to equilibrate their temperature to the test room. The instrument was switched on 30 minutes before taking the first measurement to get a stationary operating performance (whether this is an adequate period will be discussed later). After this, the white and dark references were passed once manually. These references were used to standardise all the spectra taken over the next four days. During this period the spectrometer remained switched on and spectra were measured on the compound feed cell at regular intervals (½ hour ~ 1 hour) throughout the working day. The absorbance value (log 1/ R) for each optical reading at each wavelength λ_i was calculated as follows:

$$\log(\frac{1}{R}) = \log\left(\frac{R_{white} - R_{dark}}{R_{sample} - R_{dark}}\right), \quad (1)$$

Where:

R_{white} = Intensity of the remitted radiation for the instrument white standard.

R_{dark} = Intensity of the remitted radiation for the instrument dark standard.

R_{sample} = Intensity of the remitted radiation for the sample cell.

For each measurement the Haldrup gyratory platform was rotated down to a stable position (Figure 1a) where the spectrometer measurement window was 30 mm above the sample cell, always above the same point. After the measurement the instrument was rotated back up to its other stable position (Figure 1b), to avoid the light source affecting the sample cell. During these tests, the laboratory temperature was nearly constant around 19 ~ 20 ºC but the atmospheric humidity ranged between 40 ~ 50 %.

2.2) SECOND TEST SERIES

The procedure applied in these tests is similar to the former one, but in this case the experimental process was begun two days after completion of the first series (during which 48-hour period the spectrometer was switched off) by switching on the spectrometer 24 hours before making the first reference measurement. In this case, the white and dark references were passed every hour, and the compound feed cell was measured each 20 minutes (approximately) following the process previously described. There is a sequence of three sample measurements corresponding to each reference measurement, one immediately afterwards and two taken later. In addition, the white standard was also measured as if it was a sample, just after every measurement of the compound feed cell. The time employed in these experiments was about 10 hours.

Again, the laboratory temperature was nearly constant around 19 ~ 20 ºC and the atmospheric humidity ranged between 40 ~ 50 %.

3) RESULTS AND DISCUSSION

3.1) FIRST TEST SERIES RESULTS

The appearance of the spectra measured in the NIR region during the first 45 hours is shown in Figure 2, in which 36 spectra are plotted.

In order to focus on the near infrared region, and because the spectra become noisy above 1640 nm, only the spectral range 1100 ~ 1640 nm has been considered. It is notable that all spectra are very similar even though the white and dark references were only taken at the beginning. If the mean spectrum is taken as a baseline and subtracted from the others, it is possible to see the differences between the spectra. It is difficult to see all of the spectra in one plot, so Figure 3 displays some typical difference spectra. When all the differences are examined there are no obvious trends with time. Although the curves display structured variability the extent of the variation is quite small. The maximum absorbance differences between spectra are of the order of $3*10^{-3}$.

In addition to these computations, the “root mean square” deviation was also determined for this sequence of spectra. This parameter was calculated as:

$$RMS_j = \sqrt{\frac{\sum_{i=1}^{i=N} \left(Y_{ij} - \overline{Y_i}\right)^2}{N}}, \qquad (2)$$

where:

RMS_j = root mean square deviation for spectrum j

N = number of wavelengths (λ_i) in each spectrum j

Y_{ij} = absorbance for spectrum j at wavelength λ_i

$\overline{Y_i}$ = mean absorbance at wavelength λ_i averaged over J tests

Figure 4 shows the values of RMS*10^6 depending on time during the first 45 hours. The intervals with no points are due to non-working hours (mainly at night) when no measures were taken although the spectrometer was left switched on. For these tests, the mean RMS*10^6 value was around 645. Experience with repacking and remeasuring the same sample with this instrument (unpublished data) suggests that the RMS*10^6 is around 15000 in this situation. Compared with this, the variability observed here is negligible.

After 45 hours, the compound feed cell was accidentally moved a few millimeters, and the spectrum changed slightly. To avoid further problems, the sample cell was fixed more securely to the conveyor belt. During this process the computer and the spectrometer were restarted (the spectrometer light source was switched off for roughly three minutes), and the spectral measurements were continued for a further 48 hours without passing any reference.

In Figure 5 are displayed some representative absorbance differences between the spectra measured in this new period, with the mean spectrum registered during these 48 hours as baseline. As before, when all the differences are examined there is no clear trend with time. Again there is structured variability, similar to that seen Figure 3, and the overall magnitude of the variability is also very similar to that observed in the first sequence. Notable in both Figures 3 and 5 is the "V" pattern followed by the curves near the 1400 nm region. It seems probable that this is related to humidity changes in the air of the laboratory. There was a 30 mm gap between the spectrometer and the sample cell when each spectrum was taken (see Figure 1), and the laboratory humidity ranged from 40 ~ 50%. These are difference spectra, so depending on the direction of the humidity change the peak can point either up or down.

Figure 6 shows the evolution of the RMS values (Equation 1) with time. As in the first sequence no measurements were made during the night. In both Figures 4 and 6, there is a sharply decreasing initial trend, then after the first few hours the RMS values vary apparently randomly around a mean level. This suggests that the warm-up time of 30 minutes at the start of the first sequence was not adequate, and that even the short power-down before the start of the second sequence was enough to interfere with the stability of the instrument, presumably via its effect on the lamp.

3.2) SECOND TEST SERIES RESULTS

This test series involved instrument calibration every hour, and the measurement of the compound feed cell and the white standard (treating it as a sample), as described in section 2.2.

For the compound feed cell experiments, if all the spectra are plotted the result looks just like Figure 2, and again it is necessary to look at differences to see the variability. Figure 7 shows the spectra for the first measurement in each set of three, i.e. the one taken immediately after the reference was passed, with the mean of all the spectra in the series taken as a baseline and subtracted. In each case the other two spectra that use the same reference are very similar to the first, and have been excluded so that the plot is clearer. The times in hours from the start of the series are shown at both ends of each spectrum. Interestingly there is a clear time trend at the 1100nm end of the spectrum, but not in the size or direction of the peaks at 1400nm, nor at the 1640nm end. The familiar patterns from Figures 3 and 5 are seen again here.

Figure 8 shows the RMS parameter *versus* time for the second test series. The points marked with two circles indicate the measuring times at which the white and dark references were also taken. The mean RMS*10^6 for this sequence is equal to 438. Comparing this result, and the figure, with those for the previous tests (RMS*10^6 of 645 and 278 respectively, and Figures 4 and 6), it is obvious that no great improvement has been achieved by passing the white and dark references each hour or so. Although there are some relatively high RMS values at the start of the series, they are no higher than those at the end, and noticeably smaller than those at the start of Figures 4 and 6.

It is obvious that any spectrometer that includes a light source should be powered up some time before taking any measurement. Some authors propose a two-hour period to avoid such errors.[7,9] Nicolaï et al.[6] applied an overnight period to ensure the instrument accuracy. However, no justification was presented in these works for the

use of these particular intervals. The RMS values presented in Figures 4, 6 and 8 cannot point to a particular optimal warm-up period, but they do suggest that 30 minutes is too short, and that 24 hours is adequate.

For the white standard taken as a sample cell, the spectra, again with the mean spectrum taken as a reference and subtracted, are plotted in Figure 9. Most of the spectra are clustered around the zero line, showing very little variability, whilst ten of them are more variable, with clear structure visible. The central cluster includes all of the measurements that were taken immediately after a reference, as well as some others. Enlarging and inspecting this central cluster, there is no sign of the time trend seen in Figure 7. The six spectra with an upwards pointing peak are the second and third measurements corresponding to the reference measurements at 1, 4 and 8 hours. The four spectra pointing downwards are the second and third measurement corresponding to the reference at 9 hours, and the third measurements only corresponding to the references at 0 and 7 hours. Given the clear peak at 1400nm in these spectra it seems fairly clear that this is an effect of humidity changes in one or other direction. Although the ceramic on which these measurements are taken is enclosed in a tube, there will be ambient air trapped in the tube when it is located on the instrument for measurement.

Finally, Figure 10 shows the RMS*10^6 values for the white standard depending on time. As before, the double circles indicate when white and dark references were also taken. The mean RMS*10^6 for these tests was close to 217, which is not much different from the values registered in previous tests. In addition, it is worth noting that the higher RMS values (around 400) are near uniformly distributed throughout the plot and

correspond exactly with those spectra in which the humidity peak is present (see Figure 9).

CONCLUSIONS

Considering the results presented in this work, it is reasonable to indicate that:

- Under a stable environment (temperature nearly constant and daily humidity changes of the order of 10%), there is not an appreciable improvement in the spectrometer accuracy if the white and dark references are taken hourly.

- For short term periods (of the order of a week), the accuracy of the instrument seems to be a little better if such an instrument is switched on 24 ~ 48 hours before taking any measure and is used uninterruptedly.

- The spectrometer type evaluated in this work could be used without an automatic mechanism to control white and dark references for “on line” applications so long as the environmental conditions are not strongly changing.

ACKNOWLEDGEMENTS

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Table 1. P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Table 1. Corona 45 VISNIR specifications

Feature	Specifications
Spectrometer	Singlebeam diode array
Wavelength range	400-1680 nm
Wavelength resolution	(400-950 nm: 3.3 nm/pixel), (950-1680 nm: 6 nm/pixel)
Wavelength accuracy	(400-950 nm: < 0.5 nm), (950-1680 nm: < 1 nm)
Noise (1s measuring time)	< 0.2% R
Light sources	Halogen lamp, 10V /18W
Lifetime of the light sources	approx. 2000 h
Measuring head	0° / 45° circular
Range of operating temperatures	0-40°C
Measuring rate	maximum 50 measur./second with RS 422 Interface
Calibration period	< 1h

Figure 1.a: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 1.a: Spectrometer in measuring position

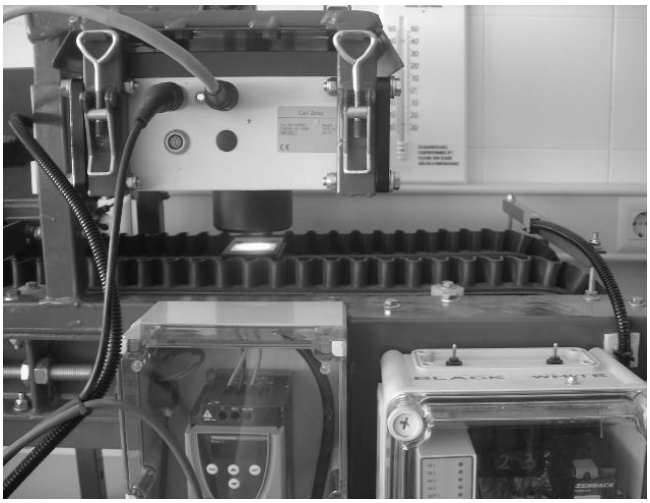

Figure 1.b: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 1b: Spectrometer in non-measuring position

Figure 2: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 2: NIR spectra measured during 45 hours, on the same point, only passing white and dark references at the beginning

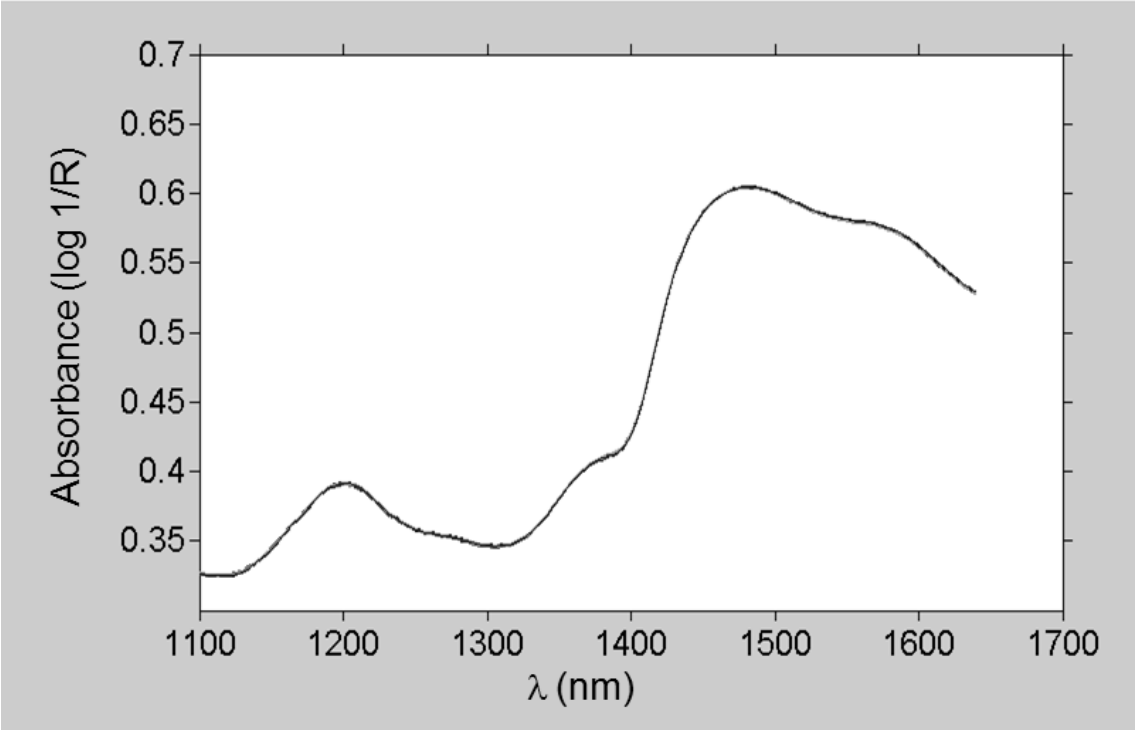

Figure 3: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 3: Spectral differences during 45 hours in the first test series

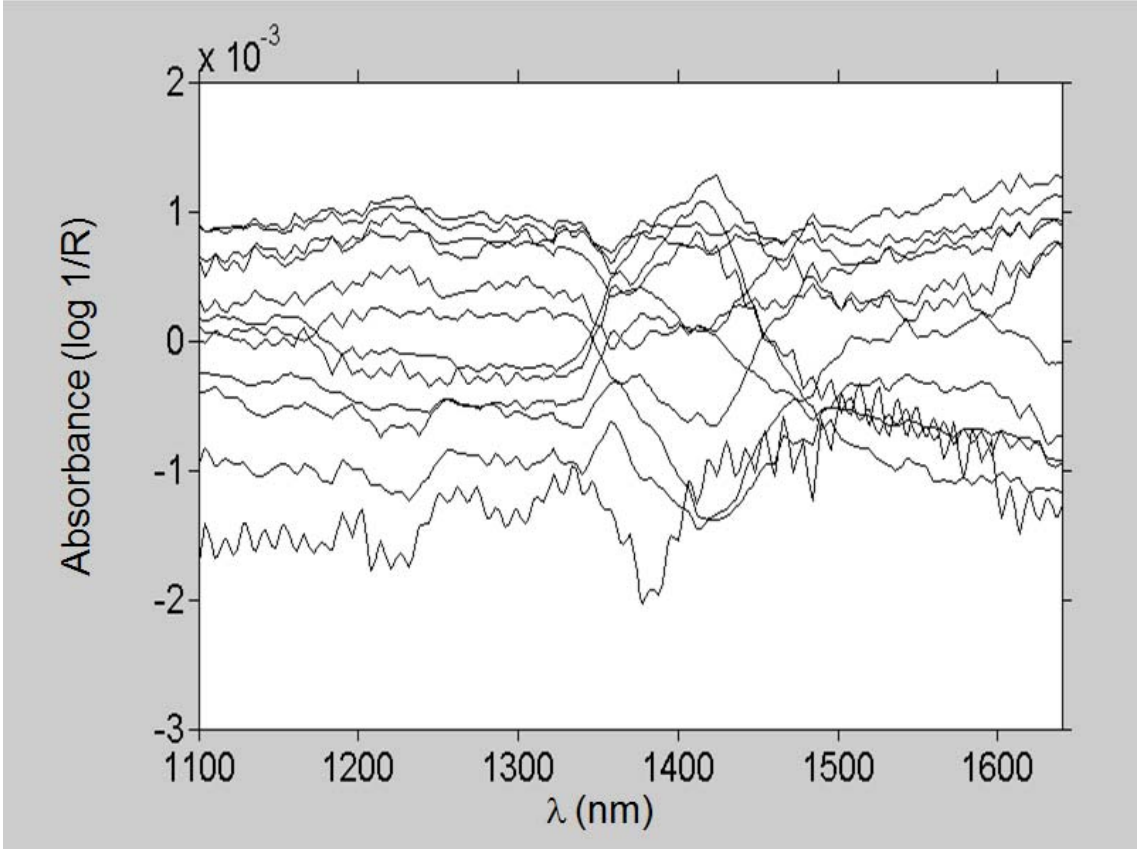

Figure 4: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 4: RMS values during 45 hours in the first test series

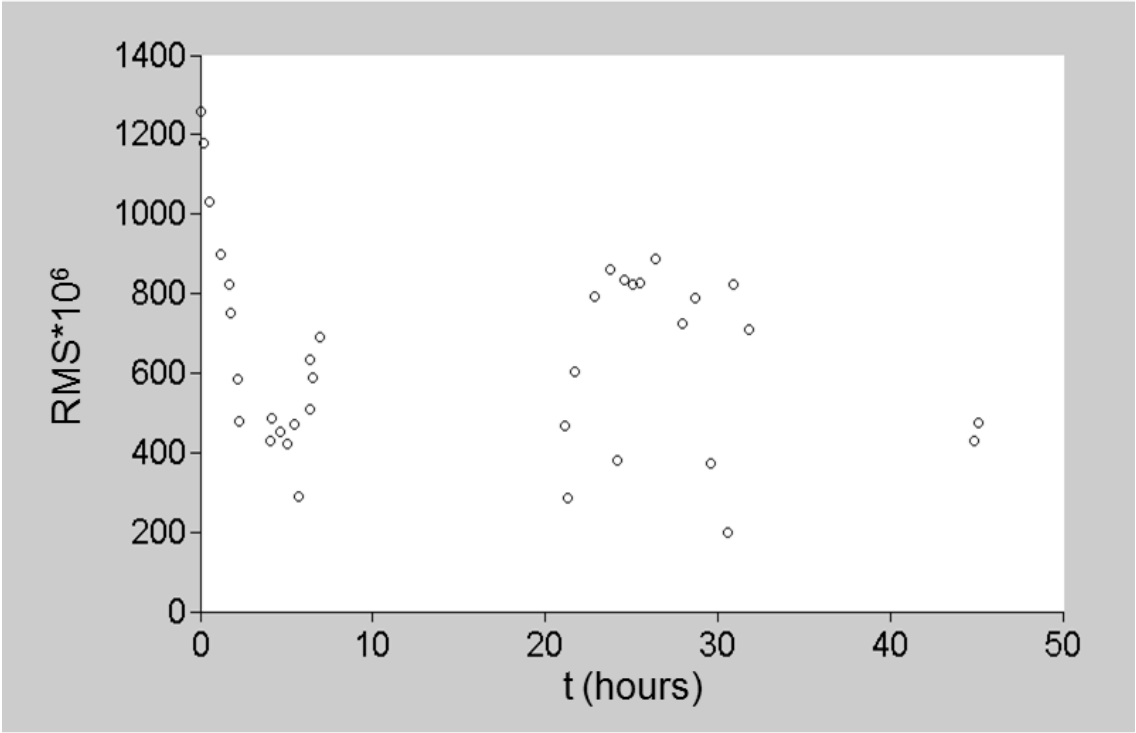

Figure 5: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 5: Spectral differences over a second 48 hours period

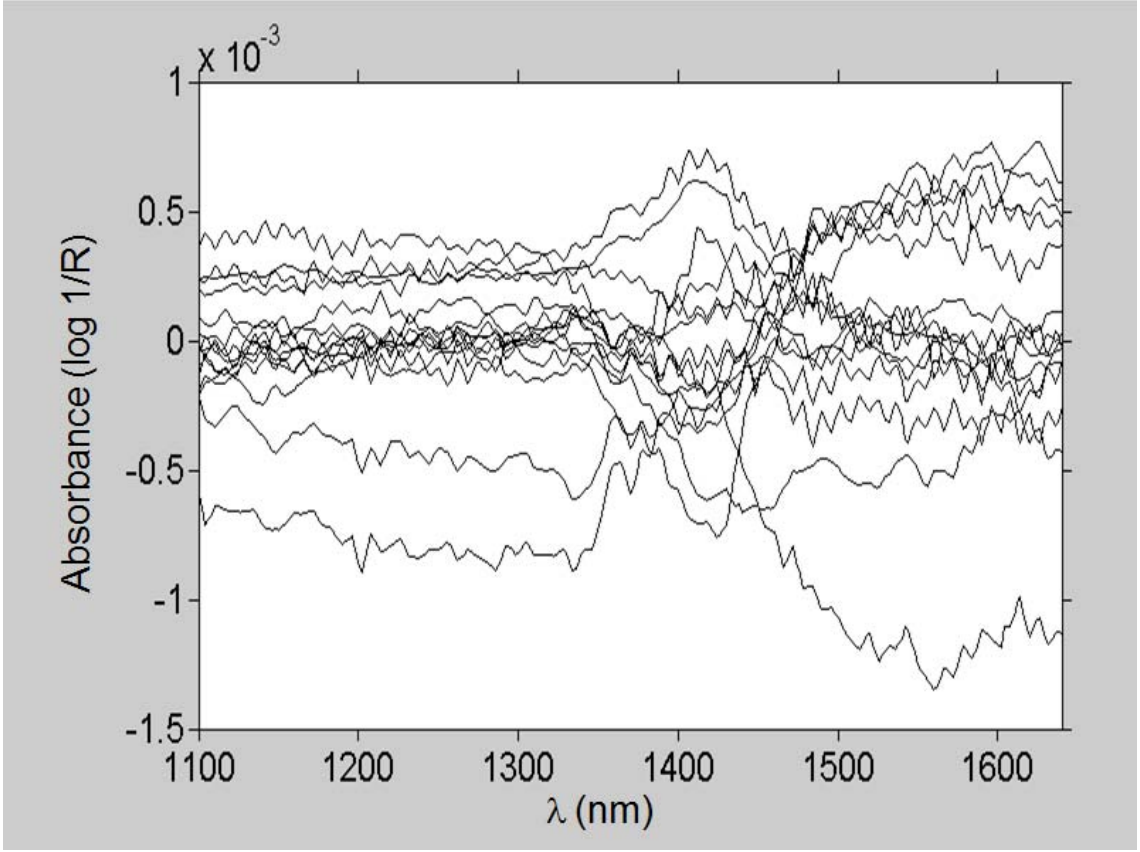

Figure 6: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 6: RMS values for the second 48 hours period

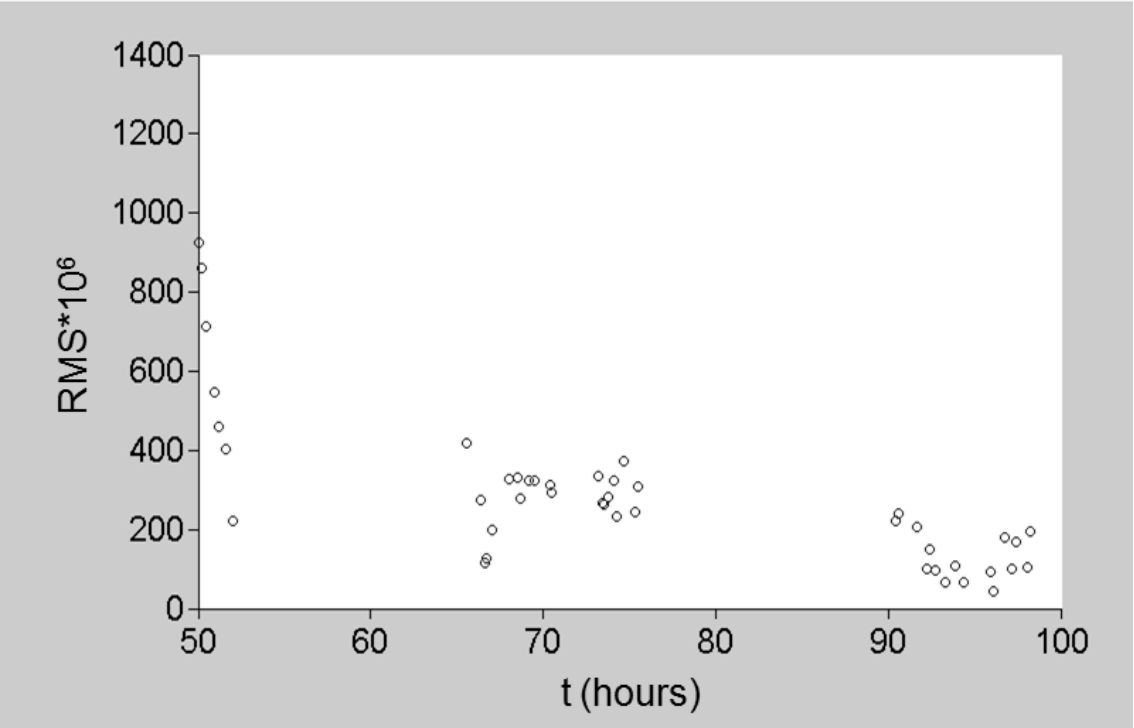

Figure 7: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 7: Spectral differences during the second test series

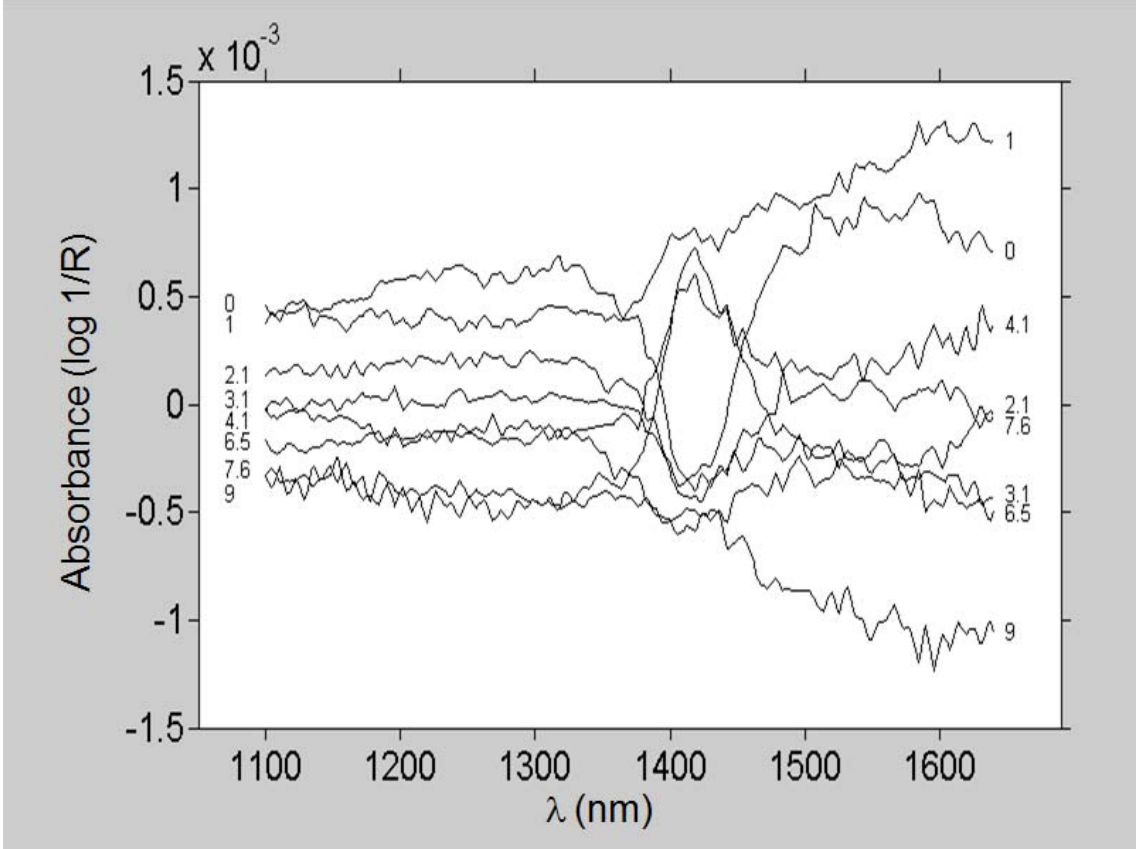

Figure 8: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 8: RMS values for the second test series

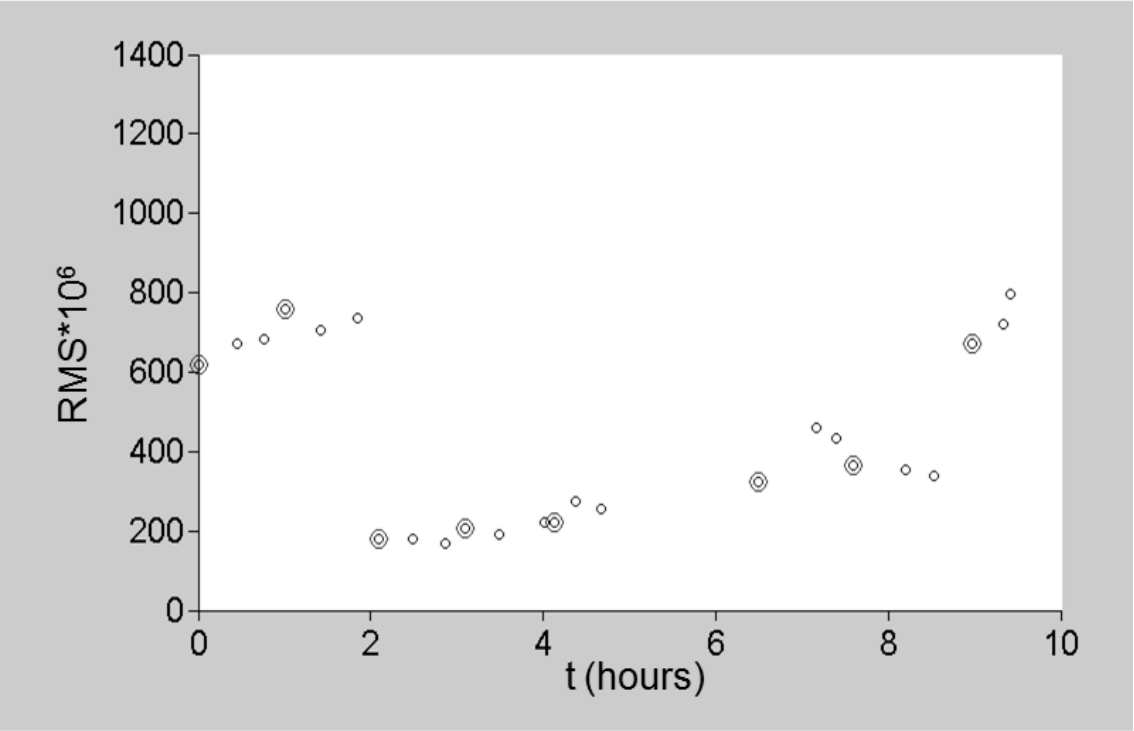

Figure 9: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 9: Spectral differences for the white standard cell

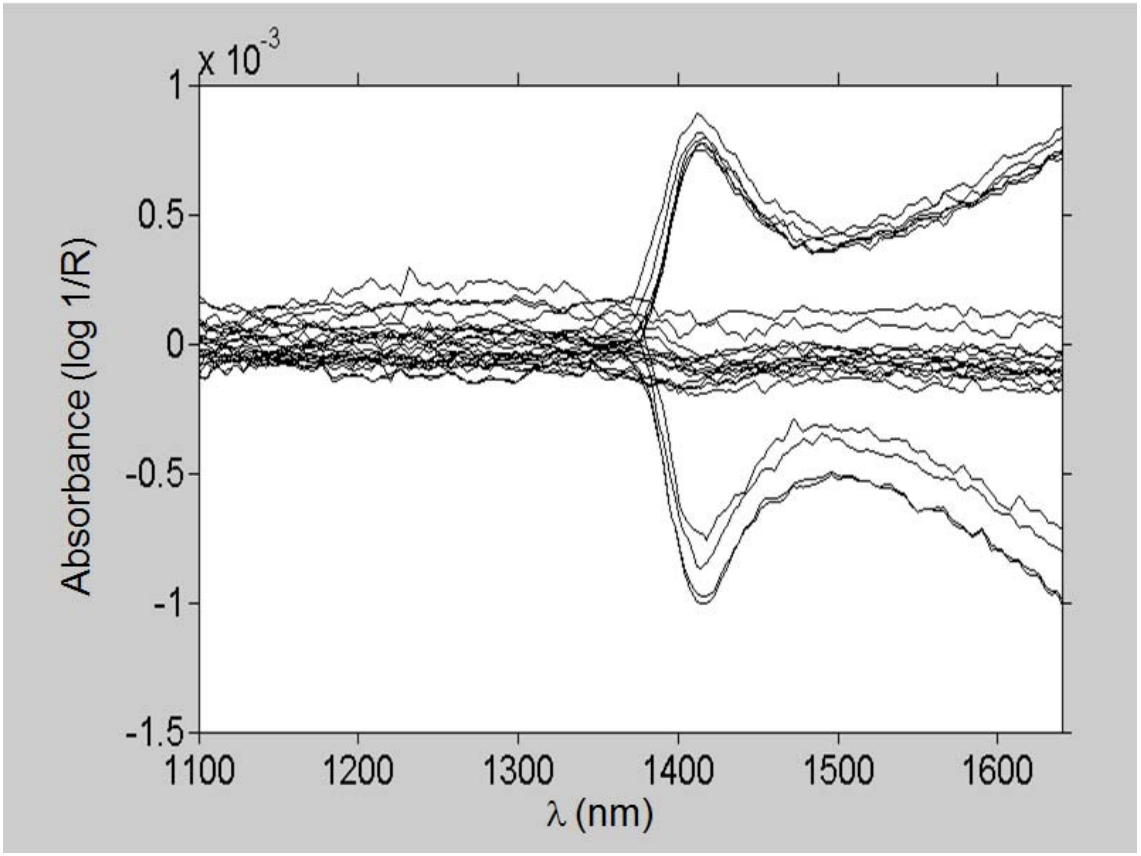

Figure 10: P. Vallesquino-Laguna, T. Fearn, A. Garrido-Varo, E. Fernández-Ahumada, D. Pérez-Marín, J. E. Guerrero. How often do references need to be measured when using a near infrared diode array spectrometer.

Figure 10: RMS values for the white standard cell