Technical Note: 51696

Advanced Near IR Algorithm Compensates for Spectral Features Related to Changes in Sampling Vials

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Key Words

- Method Transfer
- Quantitative Analysis
- TQ Analyst
- Transfer Standards

Abstract

Low cost, disposable culture tubes are often used for the rapid analysis of liquid samples by Fourier transform near-infrared (FT-NIR) spectroscopy. An evaluation of tubes from two suppliers revealed significant differences in the FT-NIR spectra obtained from the empty tubes. A quantitative analysis technique developed at Sandia National Laboratory provides a simple way to augment a classical least squares (CLS) with a principal component regression method to compensate for spectral variance not accounted for in the original standards. This augmented classical least squares (ACLS) technique should also prove valuable for creating robust methods for transfer among multiple instruments.

Introduction

FT-NIR spectroscopy has become a routine analytical technique for determining component concentrations in a broad range of chemical and pharmaceutical products. To reduce the cost and improve the speed of analysis, liquid samples are frequently measured in disposable culture tubes. Figure 1 shows a high performance instrument configured to analyze liquids by FT-NIR spectroscopy.

Classical least squares (CLS) was one of the first multivariate methods used to determine component concentrations from infrared and near-infrared spectral data. The CLS model is based on the assumption that a mixture spectrum can be approximated by a linear combination of the spectra of the pure components. This approach works well if all spectral features are related to the defined pure components. If significant features in the spectra of the unknown samples are not defined in the pure component spectra, the component concentration values the method calculates may be inaccurate. In many applications, a major limitation of CLS methods is the inability to compensate for spectral variance that is unrelated to the pure component spectra. However, David M. Haaland, David K. Melgaard and co-workers at the Sandia National Laboratory frequently use the CLS multivariate calibration technique because it allows for better qualitative interpretation of the spectra acquired from the unknown samples.

Figure 1: Antaris FT-NIR spectrometer configured for liquid analysis

In many applications, more sophisticated modeling techniques such as partial least squares (PLS) and principal component regression (PCR) provide significantly better prediction accuracy when some of the variance in the spectra of the unknown samples is unrelated to the components the method is set up to measure. However, the PLS technique lacks a clear explanation for the direct cause-and-effect relationship between the sample spectra and the quantitative result. This may cause concern when transferring a method to another instrument, or when a spectral change occurs that is not related to the analyte concentration. If new calibration spectra defining the change are added to the method, the spectra for the new calibration standards may have new features, causing the PLS loading spectra to be completely different.

This paper describes the use of an augmented classical least squares (ACLS) algorithm to correct an analytical method that measures the concentration of water in ethanol for a change in the supplier of the disposable sampling vials. The ACLS method, as implemented in Thermo Scientific TQ Analyst™ method development software, allows the developer to add standards, using a principal component regression of the residual, that can be used to compensate for any spectral variance related to the difference in sampling vials while retaining all the information in the original method.

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Theory

The mathematical basis for the augmented classical least squares method is straightforward. The method contains two sets of calibration standards: method standards and transfer standards. The method standards define the components the method is designed to measure by calculating the pure components and then augmenting to describe other sources of variability in the method standards. The transfer standards describe any spectral variation that is present in the transfer samples, but not represented by the method standards.

The software uses the spectral and concentration information from the method standards to create a classical least squares calibration model. Then it uses the model to calculate the residual of the method standards spectra according to the equation:

$$E_a = A - KC$$

Where **K** has **p** rows that correspond with the number of wavenumber intervals in the selected spectral region, and **c** columns that correspond with the number of components.

The software calculates two sets of mathematical shapes (vectors) for augmentation. The first set of shapes is based on the residual error E_a from the method standards. This produces a K_s matrix with the number of rows corresponding to **p** (the number of wavenumber intervals) and with the number of columns corresponding to the number of components plus the number of additional shapes. The optimum number of additional shapes is determined by minimizing the error of cross validation.

The second set of shapes is derived from the residual error from the transfer standards. The optimal number of additional transfer shapes is also determined by minimizing the error of cross validation of the transfer standards. This produces a K_t matrix with the number of rows corresponding to **p** (the number of wavenumber intervals) and with the number of columns corresponding to the number of components plus the shapes from the method standards and the additional shapes from the transfer standards.

Then it uses the model to predict the component concentrations of an unknown sample according to the following equation:

$$C_{unk} = [K_tTK_t]\text{-}1K_tTA_{unk}$$

Experimental

We made the samples and standards used in this example method by adding small amounts of water to pure ethanol to create a series of mixtures containing up to 10% water. We collected the spectral data from the transmission module on a Thermo Scientific Antaris™ FT-NIR analyzer. When collecting the spectra, we made no effort to control the temperature of the samples. We acquired all the data at 8 cm^{-1} spectral resolution using a measurement time of 0.5 minutes per sample.

We used the series of mixtures described above and similar sampling conditions to collect two sets of standards:

- Standard set 1, measured using disposable culture tubes purchased from Fisher Scientific, Nepean, Ontario, Canada.
- Standard set 2, measured using disposable culture tubes supplied by Kimble Kontes, Vineland, NJ, U.S.A.

To demonstrate the advantages of the ACLS calibration technique over the CLS technique when additional variability appears in the unknown samples, we created a traditional CLS method and calibrated the method in four stages:

Calibration 1

CLS calibration technique using part of standard set 1 for method standards and the rest of standard set 1 for validation standards to verify the method's initial performance.

Calibration 2

CLS calibration technique using standard set 1 for method standards and part of standard set 2 for validation standards to show the method's performance with the Kimble culture tubes.

Calibration 3

ACLS calibration technique using standard set 1 for method standards, no calibration transfer standards, and part of standard set 2 for validation standards. These calibration values allow you to compare the ACLS technique to the CLS technique using the same calibration and validation standards.

Calibration 4

ACLS calibration technique using standard set 1 for method standards, 3 standards from standard set 2 for calibration transfer standards, and the rest of standard set 2 for validation standards. These calibration results show the ACLS method's performance when transfer standards are added to the calibration matrix.

After each calibration, we recorded the root mean square error of calibration (RMSEC) value and the root mean square error of prediction (RMSEP) value. TQ Analyst software uses all the calibration standards, including any calibration transfer standards, to calculate the RMSEC value. The software uses the full calibration model and the validation standards to calculate the RMSEP value. We also recorded the number of shapes, if any, used for calibration, including any transfer shapes.

We used the Analysis Type parameter on the Description tab in TQ Analyst software to specify the CLS or ACLS calibration technique. For the CLS method, all the standards were listed in the Standards table on the Standards tab. For the ACLS method, the method standards were included on the Standards tab and the transfer standards were placed on the Transfer tab. We used the Usage parameter in TQ Analyst software to define the status of each standard (Calibration, Validation, Correction, or Ignore).

Note – The Transfer tab shows up in TQ Analyst software only when the ACLS analysis type option is selected.

For Calibration 1, we set the Analysis Type to CLS, imported the Set 1 standards, set the Usage for 10 of the standards to "Calibration," set the Usage for the remaining 3 standards to "Validation" and then calibrated the method. Figure 2 shows the calibration results.

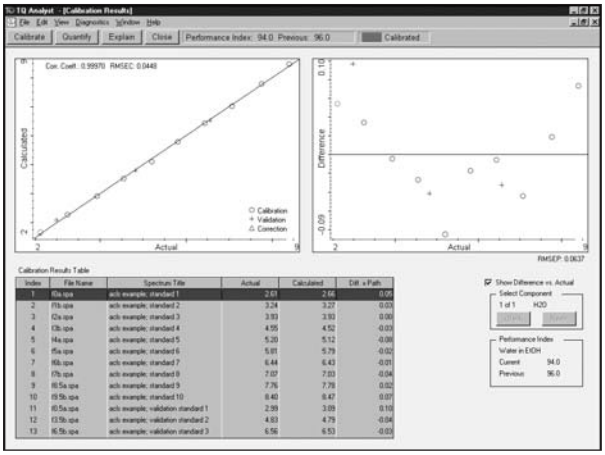


Figure 2: Calibration results for CLS method with 10 calibration spectra and 3 validation spectra, all measured in culture tubes from Fisher Scientific (Calibration 1)

For Calibration 2, we left the Usage of the Set 1 standards set to "Calibration," imported the Set 2 standards, set their Usage to "Validation" and recalibrated the method. The calibration results (see Figure 3) allowed us to compare the CLS method's performance with samples measured using the same type of culture tubes used for calibration to its performance with samples measured using culture tubes from a different vendor.

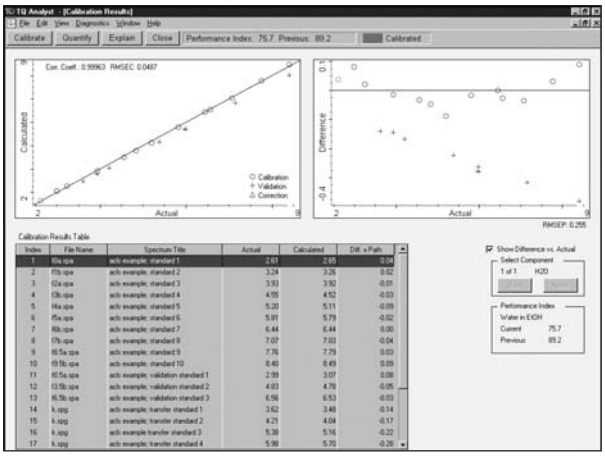
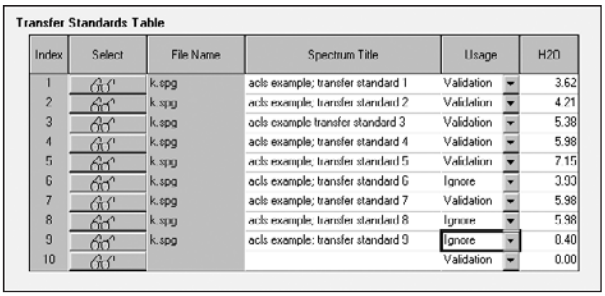


Figure 3: Calibration results for CLS method with 13 calibration spectra measured in culture tubes from Fisher Scientific and 6 validation spectra measured in culture tubes from Kimble (Calibration 2)

For Calibration 3, we changed the Analysis Type to ACLS, moved the Set 2 standards to the Transfer tab, set the Usage for three of those standards to "Ignore" so they would not be used for calibration or validation, set the Usage for the remaining Set 2 standards to "Validation" as shown in Figure 4, and recalibrated the method.



Transfer Standards Table

Index	Select	File Name	Spectrum Title	Usage	H20
1		k.spg	acls example; transfer standard 1	Validation	3.62
2		k.spg	acls example; transfer standard 2	Validation	4.21
3		k.spg	acls example transfer standard 3	Validation	5.38
4		k.spg	acls example; transfer standard 4	Validation	5.98
5		k.spg	acls example; transfer standard 5	Validation	7.15
6		k.spg	acls example; transfer standard 6	Ignore	3.93
7		k.spg	acls example; transfer standard 7	Validation	5.98
8		k.spg	acls example; transfer standard 8	Ignore	5.98
9		k.spg	acls example; transfer standard 9	Ignore	0.40
10				Validation	0.00

Figure 4: Transfer Standards table displayed in TQ Analyst software showing the Usage parameter settings for the calibration and validation transfer standards

The calibration results (see Figure 5) allowed us to compare the ACLS method to the CLS method using the same calibration and validation standards.

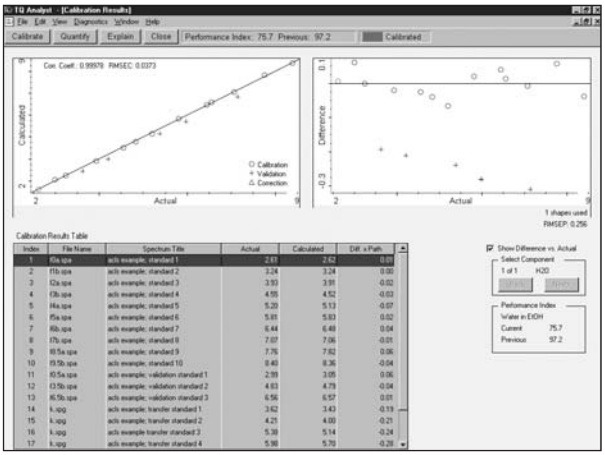


Figure 5: Calibration results for ACLS method with 13 calibration spectra measured in culture tubes from Fisher Scientific and 6 validation spectra measured in culture tubes from Kimble (Calibration 3)

For Calibration 4, we changed the Usage setting of the three unused Set 2 standards on the Transfer tab from "Ignore" to "Calibration" and recalibrated the method. The calibration results (see Figure 6) allowed us to evaluate the ACLS method's performance when transfer standards are added to the calibration model.

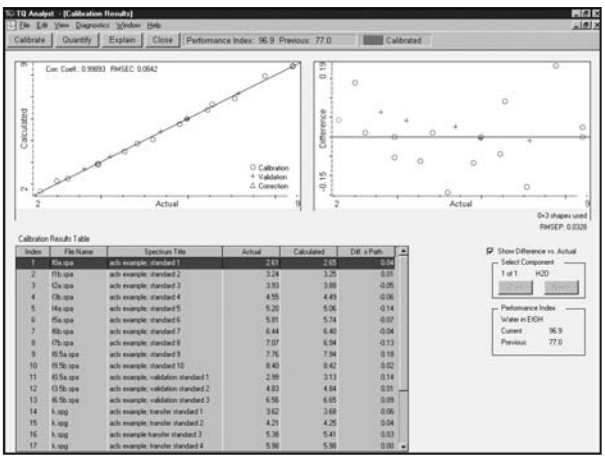


Figure 6: Calibration results for ACLS method with 13 calibration spectra measured in culture tubes from Fisher Scientific, and 3 calibration transfer spectra plus 6 validation transfer spectra measured in culture tubes from Kimble (Calibration 4)

Results and Discussion

A comparison of the spectra collected from the two types of culture tubes revealed obvious differences. The top spectrum in Figure 7 shows the average of five spectra collected from five empty tubes supplied by Kimble. The middle spectrum shows the average of five spectra obtained from five empty tubes supplied by Fisher Scientific. The lower spectrum in Figure 7 shows the difference between the two average spectra. While the spectral differences are less than 0.002 absorbency units, the peak near 7000 cm^{-1} is in the region used to calibrate for water.

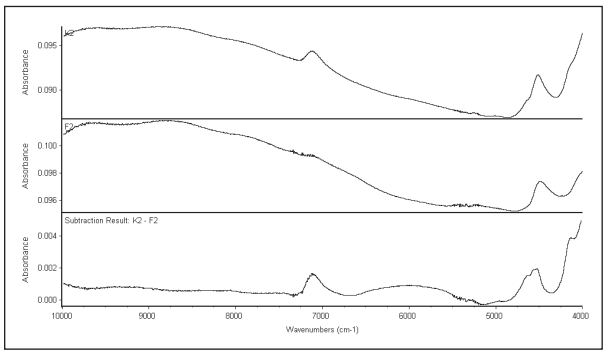


Figure 7: Comparison of spectra collected from two kinds of culture tubes. Average of spectra collected from five culture tubes supplied by Kimble (top), average of spectra obtained from five tubes supplied by Fisher Scientific (middle), and subtraction result (bottom)

Figure 2 shows the RMSEC value (0.045% water) and RMSEP value (0.064% water) for the CLS method when all the calibration and validation standards are measured in culture tubes from Fisher Scientific. When we used the CLS method to predict the concentrations of samples measured in culture tubes supplied by Kimble (see Figure 3), the error of prediction (RMSEP value) was four times higher (0.24% water). This indicates the difference in the tubes affected the prediction accuracy of the method.

Figure 5 shows the calibration results for the ACLS method calibrated with the same standards as above (13 calibration standards in tubes from Fisher Scientific and 6 validation standards in tubes from Kimble). The RMSEC value is slightly lower (0.037% water) than the corresponding value for the CLS method because we added a shape vector to the method, and the RMSEP value is slightly higher (0.256% water).

When we used several of the spectra measured in the Kimble tubes as calibration transfer standards, the ACLS method performed significantly better (see Figure 6). The calibration results show that the software added two shapes based on the spectral variance found in the spectra measured in the Kimble culture tubes. It is interesting to note that the error of calibration (RMSEC value) is higher (0.084% water). This occurred because we added three spectra with very different spectral features. However, the RMSEP value for the same six validation spectra used earlier is significantly lower (0.029% water) due to the two shapes added to the prediction matrix. Table 1 summarizes the results of the four stages of method calibration.

Calibration	Technique	RMSEC	RMSEP
1	CLS	0.0448	0.0637
2	CLS	0.0487	0.242
3	ACLS (no transfer standards)	0.0373	0.256
4	ACLS (with transfer standards)	0.0841	0.0291

Table 1: Calibration results

Conclusion

The ACLS calibration model significantly improves the prediction results for samples measured in culture tubes that are different from the culture tubes used to develop the original method. In this example, three transfer standards describing two additional shapes provided sufficient information to account for the change in culture tubes. The spectral and concentration information from the transfer standards is stored in a separate library file created by TQ Analyst software, leaving the original method and standards unchanged.

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