Application Note: 50696

Validated Transfer of a Working Food Method from a Dispersive Instrument to the Antaris FT-NIR Analyzer

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Key Words

- Antaris
- Food
- FT-NIR
- Method Transfer
- PAT

Introduction

With the impact of initiatives like Process Analytical Technology (PAT) in the pharmaceutical industry, the ability to transfer methods without re-running calibration standards is of paramount importance in Near-IR spectroscopy. NIR analytical methods, which are typically developed in research or QC labs, can be readily migrated out to multiple production sites. If, however, the methods do not transfer, i.e. give the same predicted numbers from one spectrometer to another, time-consuming re-collection of standards is required for each instrument where the method is implemented. Re-scanning of all standards in a method is so time consuming that it quickly becomes prohibitive when there are many methods involved, greatly reducing the value of Near-IR as a QC tool. For this reason, method transfer has been an active area of research for many years.

The hardware configuration of a spectrometer can have a significant impact on the success of a method transfer. Two of the most popular types of Near-IR spectrometers are Fourier transform (FT) and dispersive. FT instruments use a Michelson interferometer to sample multiple wavelengths simultaneously while dispersive instruments use a grating to split light into its component frequencies and scan them sequentially resulting in longer scan times and lower sensitivity. Other advantages of FT over dispersive are higher throughput (Jacquinot's Advantage), superior wavelength accuracy (Connes' Advantage), and mechanical simplicity.

Fourier transform instruments have replaced dispersive as the predominant technology for Mid-IR spectroscopy and the same technology migration is now occurring in the Near-IR. As the current fleet of dispersive instruments ages and customers look to FT-NIR for solutions, there will be a pronounced need to transfer methods from dispersive instruments to the FT instruments. The vast majority of method transfer studies, however, deal with transfer within the same hardware configuration. There is very little published work on the transfer of NIR methods from one hardware platform to another.

There are many factors to consider when migrating a method from one platform to another. Hardware differences are of primary concern in method transfer because optical differences generally lead to changes in spectral data. Other considerations are different data spacing from nm to cm^{-1}, wavelength accuracy, band shape, software and quantitative analysis packages. The current study examines and addresses these issues using a real method transfer case. In the current study, a food method and calibration built on a dispersive spectrometer is transferred with minimal effort to a Thermo Scientific FT spectrometer.

Experimental

Baseline Calibration – Dispersive method development and sampling were done on a FOSS NIR dispersive spectrophotometer with associated software. Data files containing spectral and concentration information for 307 calibration standards (Food powder) collected in the wavelength domain were interpolated to convert wavelength data (nm) into wavenumber data (cm^{-1}). These spectra were then added to TQ Analyst™, our chemometric software, as calibration standards in a new method referred to as the "baseline method". The same method parameters from the dispersive method were used in the creation of the baseline method with two exceptions (See Table 1 in Results and Discussion). Although great care should be taken to reproduce the original method in its true form, some aspects of a method may need to be altered to retain the integrity of the method or to match differences in algorithms between chemometric packages. Changes like these must be minimized, although, in some cases they are unavoidable.

Transfer Calibration – A set of 25 standards was run on a Antaris™ FT-NIR analyzer using the Sample Cup Spinner for diffuse reflectance sampling. The collection parameters were 32 scans per sample (taking approximately 25 seconds) at a resolution of 2.0 cm^{-1} with no zero filling and Norton-Beer medium apodization. These 25 spectra were also reprocessed at 8 cm^{-1} resolution to look for resolution-based effects in the data but the performance of the transfer method was unchanged. The number of scans was the same as in the previously collected 307 calibration standards from the dispersive method. Laboratory-derived component concentration information (i.e. primary number data) for this set of 25 standards was provided by the customer. Ten of the above standards were added into the baseline calibration as inoculation standards producing a transfer calibration. The remaining 15 standards were used to validate the performance of the transfer method (see Table 5).

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Validation Testing – The same 25 standards run on the Antaris analyzer and used in making the transfer calibration (*vide supra*) were sent back to the customer and run on the dispersive instrument. The predictions from the dispersive method were then compared to the predictions from the baseline method. Both sets of predictions were also compared to the laboratory primary number data. This procedure is designed to show how close the dispersive method is to the baseline method in predicting the component concentrations for an identical set of samples.

Results and Discussion

Baseline Calibration – Two files from the customer's dispersive method, one containing spectral information in a text format, one containing concentration information, were converted into JCAMP-DX format and incorporated as calibration standards in a new quantitative method (referred to as "Baseline") using TQ Analyst. During this process, evenly spaced wavenumber datapoints were interpolated, resulting in spectra in the frequency domain that had a consistent data spacing. The method parameters for the baseline method were taken directly from the dispersive method with the exception of two parameters. The analysis region in the dispersive method was 9090 cm^{-1} to 4000 cm^{-1} but because of a spectral artifact in the dispersive data, this region was truncated to 8800 cm^{-1} to 4100 cm^{-1} for the baseline calibration. The derivative smoothing function was also changed from the dispersive method where the Norris segment and gap were 4 and 4, respectively, to a segment of 5 and a gap of 4. A comparison of method specifications from the dispersive and baseline methods can be seen in Table 1.

	Dispersive Method	Baseline Method
Method Type	Partial Least Squares	Partial Least Squares
Pathlength	Standard Normal Variate	Standard Normal Variate
Regions	9090 cm^{-1} to 4000 cm^{-1}	8800 cm^{-1} to 4100 cm^{-1}
Pretreatment	First Derivative	First Derivative
Smoothing	Norris 4,4	Norris 5,4
Factors	12, 12, 11, 9	12, 12, 11, 9

Table 1: Method parameters from the customer's dispersive method and from the baseline method

Data for one or more components of many of the 307 original calibration standards were not specified. Forty three entries were unspecified for Component A, Component B, 18, Component C, 22 and Component D, 38. Using TQ Analyst's "Missing Data" algorithm, any missing data can be selectively ignored in the calibration without having to ignore entire standards. The baseline method was calibrated with the parameters and standards from the dispersive method taking into account all missing values.

To show equivalency between the dispersive method and the baseline method (i.e. that they predict the same values) 25 validation standards were run on the dispersive instrument. The 25 spectra were quantified against both the dispersive method and the baseline method. The absolute value of the difference between the predicted numbers (baseline minus dispersive methods) was then divided by the dispersive method numbers and expressed as a percentage. The resulting numbers indicate the difference in predictive ability of the baseline method from the dispersive method and are shown in Table 2. The average across all four components is 2.7%. This means that upon building a new method using TQ Analyst we have reproduced the predictive ability of the customer's original method to within 2.7%.

	Component A	Component B	Component C	Component D
Average	3.93	1.42	4.07	1.43
Average (All Components)		**2.71**		

Table 2: Percent difference between the absolute value of the dispersive method predictions and baseline method predictions divided by the dispersive method predicted numbers

Transfer Calibration – In some cases, when a method transfer is particularly challenging, the inclusion of inoculation standards (also called "transfer standards") into a calibration may help account for unwanted variability. For example, transferring a method from a dispersive to an FT instrument brings a significant change in optical hardware. By running samples on the target instrument (the one to which you are transferring the method) and including them in the baseline calibration, TQ Analyst™ can disregard spectral variance that is not related to the components of interest. This "inoculation" procedure requires running a small number of standards (about 5% of the number of calibration standards) to effectively account for the transfer to the target instrument.

Twenty five standards were run on the Antaris analyzer as potential inoculation standards. Ten were chosen for incorporation into the baseline calibration as inoculation standards and the method was re-calibrated. No other parameters were changed from the baseline method.

Usually, inoculation standards are chosen to be representative of the calibration range for the components, however, in this case, the 10 inoculation standards spanned a much more limited range. This demonstrates a very practical solution for inoculation. Due to the time and resource restraints that would be involved with finding a set of inoculation standards that had the desired component concentrations, we were able to account for the variability between a dispersive and an FT instrument using standards that were simply taken off a shelf and run without any pre-screening.

In order to compare the performance of the baseline and transfer methods, the same 25 dispersive spectra used to validate the baseline method were quantified using the transfer method. Validation of separate methods using the same spectra is extremely important in method transfer because it is an excellent gauge of relative performance. If the two methods are indeed the same, then the validation spectra should give the same predicted concentrations using either method. The same spectra were also used to validate the original dispersive method to extend our cross-method validation to include the customer's original method.

The calibration curve and residual for the transfer calibration which includes the 307 original calibration standards (run on dispersive), 10 inoculation standards (run on Antaris analyzer) and 25 validation standards (run on dispersive) are shown in Figures 1 and 2. The calibration and inoculation standards are shown as circles and the validation standards are shown as plus signs (+). The distribution of the 25 validation standards is homogeneous with respect to the calibration curve, indicating a well-represented calibration.

A comparison of the relative predicted values for the 25 dispersive spectra across the dispersive, baseline and transfer methods is shown in Figure 3. Figure 3 shows the predicted values in relative proportion indicating that the difference in predictive ability between the three methods is minute.

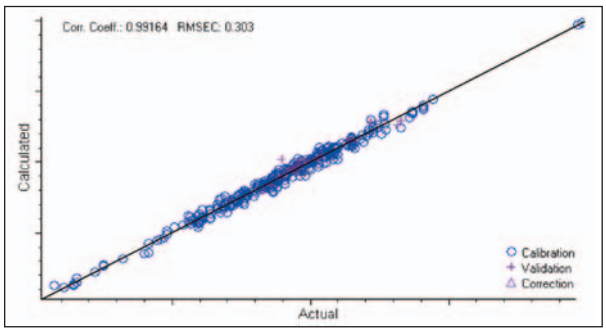

Figure 1: Transfer calibration curve for Component B including 10 Inoculation standards run on the Antaris analyzer and 25 Validation standards run on the dispersive instrument

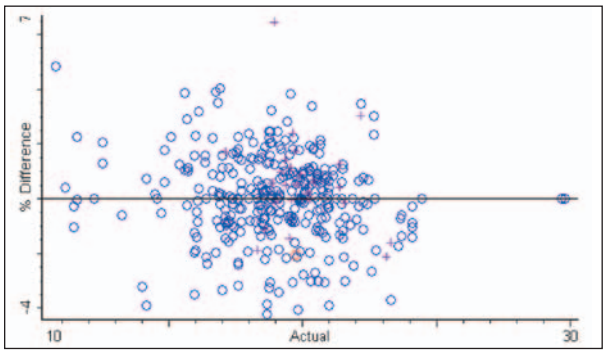

Figure 2: Calibration curve Residual for Component B

The statistical parameters commonly used to describe the performance of a calibration are Root Mean Square of Calibration (RMSEC), R^2 (line fit) and RMSEP (Root Mean Square Error of Prediction). The R^2 value describes how well the data is fit to a line, RMSEC is used to show how close the individual calibration standards are to that line and RMSEP is an indicator of how close validation standards – standards that are not in the calibration – are to the calibration line. These three statistical markers for the dispersive, baseline, and transfer methods are shown in Table 3. The R^2 values across all three methods are almost identical, as are the RMSEC values; the RMSEC value for Component C has a difference of 0.022 from the baseline

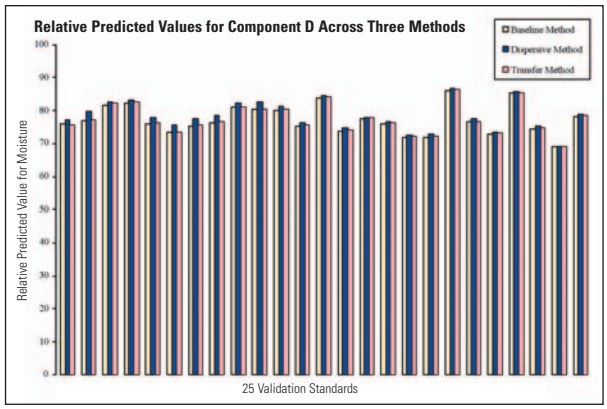

Figure 3: Relative predicted values for component D for the set of 25 validation standards quantified against the dispersive, baseline and transfer methods

method to the transfer method. The RMSEP values, predicted values that come from spectra not included in the calibration, show that, on average, the numbers predicted by the baseline and transfer methods are more precise than those from the customer's original dispersive method.

	Components			
	A	B	C	D
R^2 (Dispersive Method)	0.97	0.98	0.91	0.98
R^2 (Baseline Method)	0.96	0.98	0.90	0.97
R^2 (Transfer Method)	0.96	0.98	0.88	0.97
RMSEC (Baseline Method)	0.903	0.305	0.198	0.315
RMSEC (Transfer Method)	0.911	0.303	0.220	0.319
RMSEP (Dispersive Method)	0.98	0.52	0.71	0.53
RMSEP (Baseline Method)	1.15	0.35	0.63	0.48
RMSEP (Transfer Method)	1.20	0.36	0.60	0.48

Table 3: Comparison of calibration statistics across original, baseline and transfer calibrations

Finally, we show in Table 4 that the transfer method predictive capacity for the 25 dispersive spectra is within 1% of the baseline method. The analysis in this case is the same as was done to compare the dispersive method to the baseline method – the numbers in the table are absolute values of differences between numbers predicted by the baseline method and those predicted by the transfer method, expressed as a percentage of the baseline method. The final average, across all components, shows that the transfer method predicts within 1% of the baseline method. This difference of 1% in predictive capacity shows the performance of the transfer method against a set of 25 dispersive spectra, demonstrating that the transfer method predicts dispersive spectra almost as well as the original dispersive method. But how does the transfer method predict standards from the FT instrument?

	Component A	Component B	Component C	Component D
	2.03	0.70	4.35	0.26
	0.56	0.30	1.42	0.13
	0.83	0.44	1.85	0.61
	0.73	0.53	1.94	0.36
	0.17	0.37	2.49	0.26
	0.55	0.60	3.00	0.00
	0.50	0.21	1.61	0.40
	0.21	0.50	3.37	0.26
	1.09	0.42	4.52	0.25
	0.42	0.49	4.50	0.12
	0.59	0.30	2.67	0.50
	0.91	0.26	2.38	0.40
	0.26	0.20	0.86	0.36
	1.11	0.05	1.83	0.27
	0.45	0.15	3.50	0.39
	0.80	0.05	2.00	0.39
	0.66	0.10	1.96	0.42
	0.69	0.50	3.94	0.42
	0.94	0.20	2.23	0.35
	0.86	0.40	1.42	0.00
	1.29	0.05	2.63	0.41
	1.03	0.25	1.59	0.35
	0.64	0.21	2.31	0.27
	0.91	0.22	2.56	0.29
	0.96	0.00	1.27	0.26
Average	0.77	0.30	2.49	0.31
Average (All Components)		**0.97**		

Table 4: Percent difference in prediction values between baseline method and transfer method divided by the baseline method numbers

Inoculation, in this case, helped the predictive capacity of the baseline method (see Table 5) by building variability from the FT instrument into the transfer method. Percent improvement in RMSEP (based on a subset of the original 25 inoculation standards run on the Antaris analyzer) for the four components were 41% (A), 66% (B), 34% (C) and 10% (D). In order to truly compare RMSEP of the transfer method vs. the baseline method, only 15 of the original 25 validation standards were used because the other 10 standards were already included in the transfer calibration as inoculation standards.

Component	RMSEP (Not Inoculated)	RMSEP (Inoculated)	Inoculation Improves Performance?
A	2.17	1.28	✓
B	1.40	0.47	✓
C	0.76	0.50	✓
D	0.44	0.39	✓

Table 5: Improvement in RMSEP upon inoculation of baseline method with 3% FT standards

Conclusion

The results of this study show that a method taken from a dispersive spectrometer can be readily transferred to a Antaris FT-NIR analyzer without sacrificing prediction accuracy and without re-running large numbers of standards. The baseline method predictions for 25 validation standards run on the dispersive instrument are within 2.7% of the same standards quantified against the customer's original dispersive method. This demonstrates that the baseline method has an almost identical predictive capacity as the dispersive method. When 10 inoculation standards run on the Antaris analyzer are incorporated into the baseline method, the resulting transfer method's predicted concentrations are within 1.0% of the baseline method. Predictive ability was also measured, in this case in terms of RMSEP. Method performance was dramatically improved as gauged by the percent improvement in RMSEP across all four components. These inoculation standards make up a mere 3% of the total standard count, yet enable the method to account for optical variability between the two spectrometer platforms.

What has been demonstrated is the ability to take a working method from a dispersive spectrometer and, without sacrificing predictive ability, quickly and successfully transfer it to an Antaris FT-NIR analyzer. This process was developed to reduce downtime when migrating Near-IR technology to Fourier Transform instruments. Scan time for each inoculation standard was less than 1 minute for the current study and file transfer is effectively an automated process using Thermo Scientific software. This protocol results in an accurate, seamless method transfer in a matter of minutes.

For the customer who wants to transfer a method from an older, dispersive instrument to the Antaris analyzer, the process is simple and quick. Working with your local Thermo Scientific sales and application contacts, existing spectral data files are imported into TQ Analyst software to create the baseline method. Samples for inoculation are run on an Antaris analyzer. The inoculation standards are included in the baseline calibration to make the transfer calibration and, lastly, validation standards are run to check the performance of the new method.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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