

Agilent MicroLab Quant Calibration Software: Measure Oil in Water using Method IP 426

Application Note

Environmental

Authors

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Introduction

Like other optical spectroscopy methods, FTIR can provide accurate quantitative analysis for many analytes. With the proper calibration, FTIR spectra can yield both sensitive and selective determination. Although it was once common to post process data after collection, innovative software packages, such as Agilent MicroLab PC and Agilent MicroLab Mobile, provide direct reporting of quantitative results. Now, Agilent MicroLab Quant software makes calibration and method development easier than ever.

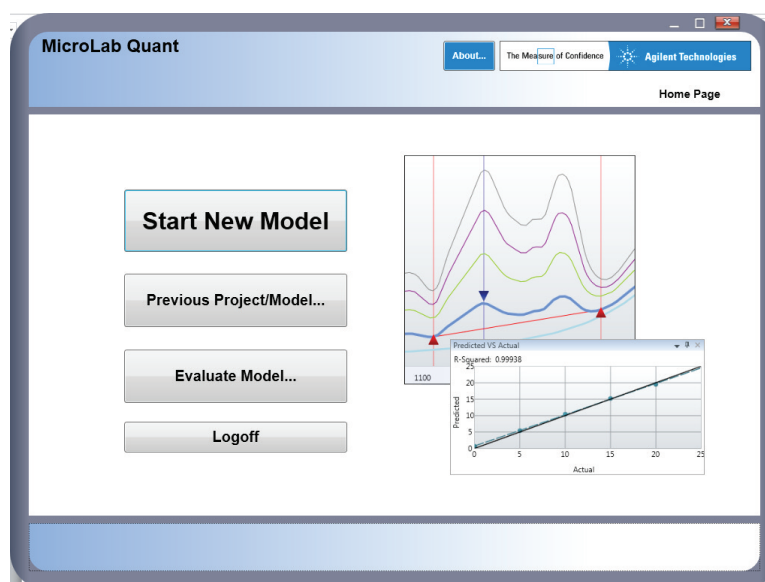


Figure 1. Agilent MicroLab Quant software develops quantitative calibrations using the same intuitive, guided user interface as industry-leading Agilent MicroLab PC software.

MicroLab Quant makes quantitative method development guided and easy-to-use, just like the other MicroLab software products (Figure 1). The software allows the development of interactive quantitative calibrations using simple Beer's law, classical least squares, or inverse least squares methods.

A simple Beer's law calibration correlates a band height, band area, or band ratio to concentrations of known standards. This is the most direct correlation and, typically, the easiest to understand. Often, a simple Beer's law method is accurate and rugged.

The classical least squares (CLS) method is similar to Beer's law except that multiple bands can be simultaneously correlated to the concentration of known standards. Definition of multiple bands within a single calibration method can provide a more accurate result, especially when nonlinearity or band overlap exists. By incorporating information from multiple bands at the same time, classical least squares taps into the power of FTIR to provide selective quantitation.

Inverse least squares (ILS) increases the level of method sophistication even further. ILS rearranges the standard Beer's law equation, making concentration the dependent variable. Through this simple change, calibrations developed using ILS can better accommodate overlapped bands and interferences by known and unknown components. By identifying some bands that correlate to the desired concentration, and other bands that are due to interfering compounds, an ILS calibration can be the most accurate and rugged of the three methods.

The procedure for method development using simple Beer's law, CLS, or ILS is identical in the MicroLab Quant software. The software guides the analyst through the selection of measured standards, band definition, method evaluation, and final output directly to a method, which can be read in the MicroLab PC software. As a practical consideration, CLS and ILS typically require more standards to work well compared to simple Beer's law. Analysts typically try a simple Beer's law calibration first. If they find difficulty, due to mixtures or band overlap, they may decide to switch to a CLS or ILS calibration. Changing between calibration types is straightforward in the MicroLab software. You can easily switch between algorithm types and directly see the results. Furthermore, more standards can be added to the calibration at any time, making the job of expanding a calibration simple, as well.

To demonstrate the use of MicroLab Quant, the standard FTIR quantitative method IP 426 for the measurement of oil in water was chosen. In this method, two bands for aliphatic hydrocarbons and another band for aromatic hydrocarbons are specified. The original method, written before the ILS technique was widely used, specifies that the oil concentration should be correlated to the sum of the two aliphatic bands minus the aromatic band. The ILS technique provides results as good or better, using a much simpler technique.

Experimental

Six standard samples of aliphatic hydrocarbons and six standard samples of aromatic hydrocarbons of 2, 10, 20, 40, 70, and 100 mg/L were prepared. Validation standards containing mixtures of aliphatic and aromatic hydrocarbons in water were also prepared. All samples were extracted in tetrachloroethylene according to IP 426. These extracts were then measured using the Agilent Cary 630 FTIR spectrometer. Measurements were conducted using a 10 mm quartz cell, as specified by the method, using the Cary 630 transmission compartment. All spectra were collected averaging 32 scans at 4 cm^{-1} resolution for a total measurement time of 13 seconds.

Results and Discussion

Calibrations

The MicroLab Quant software is designed to guide the analyst through all the necessary steps to develop a calibrated method. The analyst chooses to begin a new method from the start screen, and is then instructed to choose the calibration spectra. The software automatically selects the default results folder used in the MicroLab PC and guides the selection of files collected at the same resolution. In this example, the six spectra of the aliphatic hydrocarbon samples were chosen along with two aromatic hydrocarbon samples. Since the IP 426 method specifies bands for both the analyte of interest (aliphatic hydrocarbons) and interferences (aromatic hydrocarbons), the ILS algorithm was selected (Figure 2).

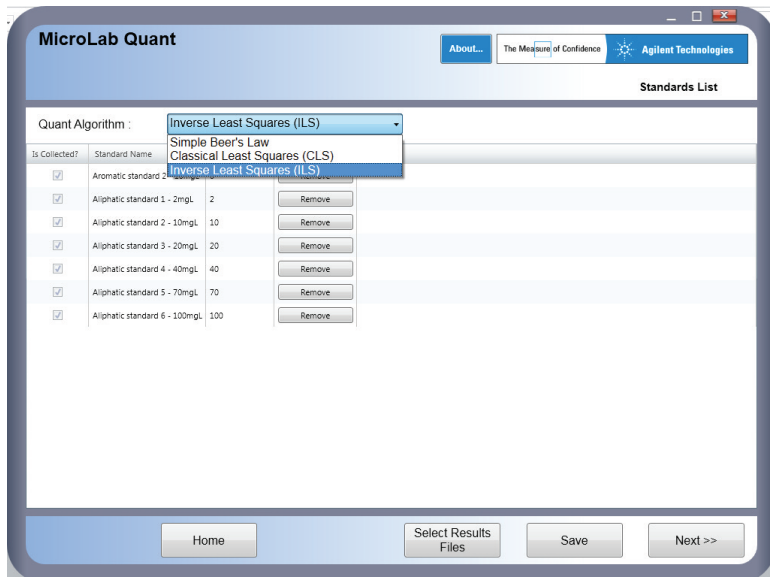


Figure 2. Concentrations of selected standards and the method type are selected on the standards screen. This screen can be accessed at any time to add or remove standards from the calibration.

The IP 426 method specifies measurement of three bands, namely the aliphatic methylene asymmetric stretch at $2,930\text{ cm}^{-1}$, the aliphatic methyl symmetric stretch at $2,960\text{ cm}^{-1}$, and the aromatic carbon hydrogen stretch at $3,030\text{ cm}^{-1}$. To define these bands, the peak height icon is simply dragged to the center of the desired band on the

spectral display (Figure 3). The software automatically assigns the center point for the peak height and two suggested baseline points. These points can be fine-tuned either graphically, by dragging the icons on the spectral display, or numerically by entering the desired values in the peak table.



Figure 3. Agilent MicroLab Quant band definition screen allows easy definition of peaks by dragging the peak icon to the appropriate location. The calibration plot in the upper right corner automatically updates, making it easy to optimize peak locations.

As the bands are defined, the calibration curve in the upper right corner of the screen updates automatically. It is possible to display the actual versus predicted plot for the entire calibration, or the peak height (area) versus concentration plot for an individual band. Bands from the calibration can be selected or deselected using the check box in the peak table display. Throughout the development process, the calibration plots and the correlation coefficients (R^2) are updated in real time, making it easy to optimize the method in real time.

Once the bands have been selected and optimized, the model

can be further tested using the Model Evaluation feature. This calculates the cross-validation results for the defined calibration. Cross validation is a technique where a single spectrum is excluded from the calibration; that spectrum is then predicted using the resulting calibration. The process continues iteratively until all the spectra are predicted. In this way, the predictions use a model that does not contain the sample being predicted. This is a more rigorous test of the method accuracy than the correlation of the calibration set. In this case, the cross validation has an exceptional correlation of > 0.9999 and a standard error of 2.17 ppm (Figure 4). These are excellent results for the large calibration range tested. Also, Model Evaluation allows testing of a separate validation set to further prove the method.

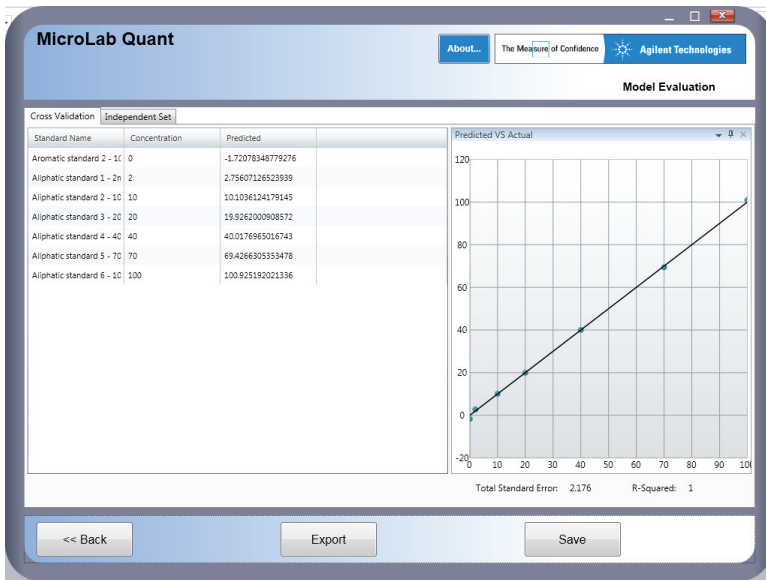


Figure 4. The Model Evaluation screen allows the calibration to be tested using both cross validation and an independent validation set. The evaluation results are saved with the Agilent MicroLab Quant project file, and can be exported to a spreadsheet.

Once a satisfactory calibration has been established, MicroLab Quant makes it easy to create a component method for use in the MicroLab PC software. The final page gives two saving options (Figure 5). The first option is to save the project and model file. The project contains all the calibration spectra, peak definitions, cross validation, and separate validation results. The project lets the analyst go back and modify or edit the calibration. In addition, a model file is saved. The model file includes the calibration information used to make a prediction in the MicroLab software. The model can be added to any new or existing MicroLab PC method file. The package can also directly save a complete MicroLab PC method containing the calibration. In addition to defining a component using the established calibration, all the data collection parameters used on the calibration files are entered into the saved method. This ensures that the new data will be collected using the same parameters of the calibration, delivering accurate predictions, and eliminating the possibility of user error.

Comparison of inverse least squares to simple Beer's law

Spectral overlap often causes inaccuracy in quantitative data. In the case of oil in water measurements, large amounts of aromatic hydrocarbon can give an erroneously high result for the aliphatic hydrocarbon as well. To combat this, IP 426 defines bands for both aromatic and aliphatic hydrocarbons. By including all three bands in the ILS calibration, the algorithm will automatically compensate for the interfering aromatic bands in the aliphatic prediction.

To demonstrate this, a simple Beer's law calibration can be established using the same spectra as the ILS calibration. A single band height at $2,930\text{ cm}^{-1}$ is defined. The cross-validation result shows a lower performance for the simple Beer's law model. The correlation value is 0.997 for Beer's law compared to > 0.9999 for ILS. The standard error is 5.60 ppm for Beers law compared to 2.14 ppm for ILS. An even more telling result is the cross-validation prediction of one aromatic standard. This sample contains no aliphatic hydrocarbon. The simple Beer's law calibration predicts the aromatic sample at 3.63 ppm while the ILS calibration predicts it at -1.7 ppm. The ILS calibration is closer to the true value of 0 ppm, and does not produce the erroneously high result.

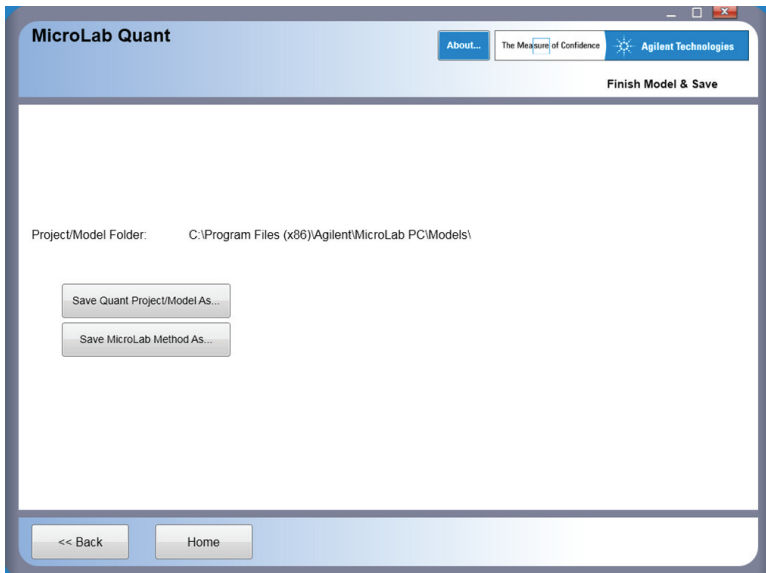


Figure 5. Agilent MicroLab Quant can save the entire project including the standard spectra, peak definitions, and evaluation data. It can also output a complete method for direct use in Agilent MicroLab PC.

Conclusions

Quantitative methods expand the capabilities of FTIR spectrometers. The sensitivity and selectivity of FTIR analysis lends itself to the production of accurate quantitative models for many substances. Agilent MicroLab Quant software makes development of quantitative methods accessible by walking the analyst through each step in the process. The software allows optimization in real time and complete evaluation of the model. Once the calibration model is built using the desired MicroLab Quant algorithm, a complete method can be saved, allowing direct use in Agilent MicroLab PC software.

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