Alcohol Levels Determination in Hand Sanitizers by FTIR

Instruction Sheet

For Research Use Only. Not for use in diagnostic procedures.

Contents

1 Scope

This application guide is designed to support the analyze of alcohol levels (ethanol and isopropanol) in hand sanitizers using the Cary 630 FTIR spectrometer with the MicroLab software as described in the “Analysis of Alcohol Levels in Hand Sanitizer” application flyer (5994-2014EN).

The document guides users through the entire method development process from preparing standard samples to creating and using a routine MicroLab method. This method allows to identify the alcohol type in the sample and automatically calculates the alcohol level.

It is the user’s responsibility to validate the method prior to using it to analyze samples.

It is also the user’s responsibility to develop, maintain, and enforce the hand sanitizer analysis SOP.

2 Safety

Check that your laboratory’s health and safety procedures include the following hazards:

- Safety glasses and acid resistant gloves should be worn at all times when handling samples or reagents, or when in the vicinity of others handling these items.
- Spilled samples and reagents should be cleaned up from instrument and laboratory surfaces immediately.

Check the Cary 630 FTIR User Guide for safety and instrument handling guidelines.

Use of the spectrometer system and accessories may involve materials, solvents and solutions which are flammable, corrosive, toxic or otherwise hazardous. Careless, improper or unskilled use of such materials, solvents and solutions can create explosion hazards, chemical burn hazards, fire hazards, toxicity and other hazards that can result in death, serious personal injury or damage to equipment. Apply all necessary precautions including use of lab coats, safety goggles and other appropriate forms of personal protection. All wastes should be disposed of in accordance with local regulatory requirements.

3 Equipment and Materials

The following equipment and materials are required for the method development following this application guide.

3.1 Materials

- Single use pipettes
- Metric flask or volumetric pipettes
- Cloth or paper wipes

3.2 Chemicals

- Distilled water
- Ethanol and/or Isopropanol
- Hydrogen peroxide (H_2O_2)
- Glycerol
- Alcohol for cleaning (ethanol or isopropanol)

NOTE The quality and purity of the chemicals used in this procedure must be equivalent to the ingredients used during hand sanitizer production.

WARNING

Chemical Hazard
Ethanol, isopropanol and hydrogen peroxide may be harmful if swallowed or inhaled. May be irritating to the skin, eyes and respiratory tract. Use only in a well-ventilated room. Place open chemicals in a fume hood or adjacent to an extraction system. Appropriate protective clothing must always be worn when handling these chemicals. If chemicals contact the skin, wash off with water.

3.3 Instrumentation

- Agilent Cary 630 FTIR spectrometer with single reflection diamond ATR sampling module
- MicroLab software

4 Solution and Instrument Preparation

4.1 Standard and independent sample preparation

It is recommended to prepare 10 standard samples with known concentration for the development of a quantification model as well as to prepare 2 independent samples with known concentration to test the created MicroLab methods.

The preparation of the standards and independent samples needs to be representative of the production of the final hand sanitizer product.

The standards and independent samples used in this document were prepared following the World Health Organization's recommendation for hand sanitizer formulations as given in Table 1.

Table 1 Standard and independent sample preparations

Formulation 1 – Ethanol based	Formulation 2 – Isopropyl alcohol based
Ethanol 80% (v/v)	Isopropyl alcohol 75% (v/v)
Hydrogen peroxide 0.125% (v/v)	Hydrogen peroxide 0.125% (v/v)
Glycerol 1.45% (v/v)	Glycerol 1.45% (v/v)
Sterile distilled or boiled cold-water remainder of the volume	

Preparation

WARNING

Chemical Hazard
Ethanol, isopropanol and hydrogen peroxide may be harmful if swallowed or inhaled. May be irritating to the skin, eyes and respiratory tract. Use only in a well-ventilated room. Place open chemicals in a fume hood or adjacent to an extraction system. Appropriate protective clothing must always be worn when handling these chemicals. If chemicals contact the skin, wash off with water.

1. For the standards: Label 10 test tubes clearly stating the final alcohol concentration.
2. For the independent samples: Label 2 test tubes clearly stating the final alcohol concentration.
3. Due to its viscosity volumetric handling of pure glycerol is not trivial. For easier handling, pre-mix the volumes of glycerol and hydrogen peroxide as required and stir the two liquids until homogenous.
4. Add the required volumes of the glycerol/hydrogen peroxide mixture to each of the test tubes (using a volumetric cylinder).
5. Add the required volumes of water to each test tube (using a volumetric cylinder or volumetric pipette).
6. Add the relevant volumes of alcohol to each test tube as required (using a volumetric cylinder or volumetric pipette). Close the lid of each test tube immediately after addition of the alcohol to prevent evaporation.

4.2 Preparing the Cary 630 FTIR

1. Connect the Cary 630 to the PC and switch it on
2. Start MicroLab PC
3. Attach the Diamond ATR module to the Cary 630 engine
4. Rotate the swivel press towards the back of the instrument to maximize access to the ATR crystal.

5 Method Development

This section includes procedures to:

- Create a method to measure the standard samples
- Measure the standard samples
- Create a quantification model using the measured standard samples
- Create a QC method for routine analysis of alcohol in hand sanitizer
- Run the QC method

5.1 Create a method to measure the standard samples

1 Start MicroLab PC or MicroLab Light

2 Click **Methods**.

3 Click **New**.

4 Click the **Type** tab.

5 Select **Data Collect Only** as the "Method Type" from the drop-down menu.

6 Set the parameters as shown in Figure 1.

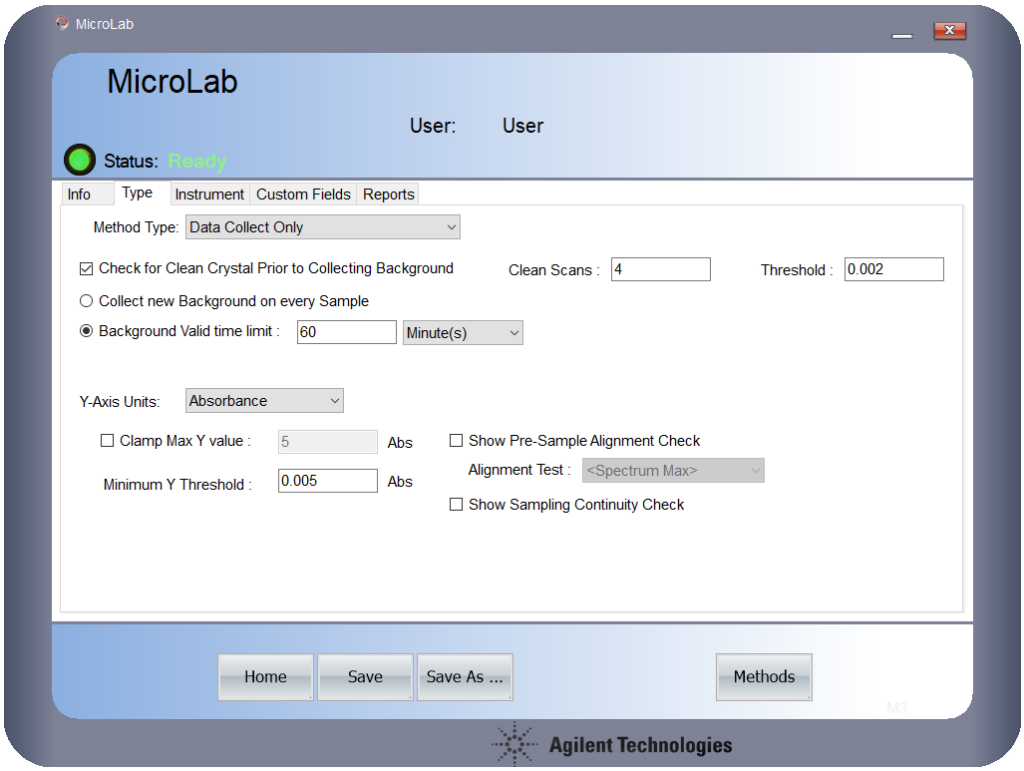


Figure 1. Method parameters

7 Click the **Instrument** tab.

8 Set the parameters as shown in Figure 2.

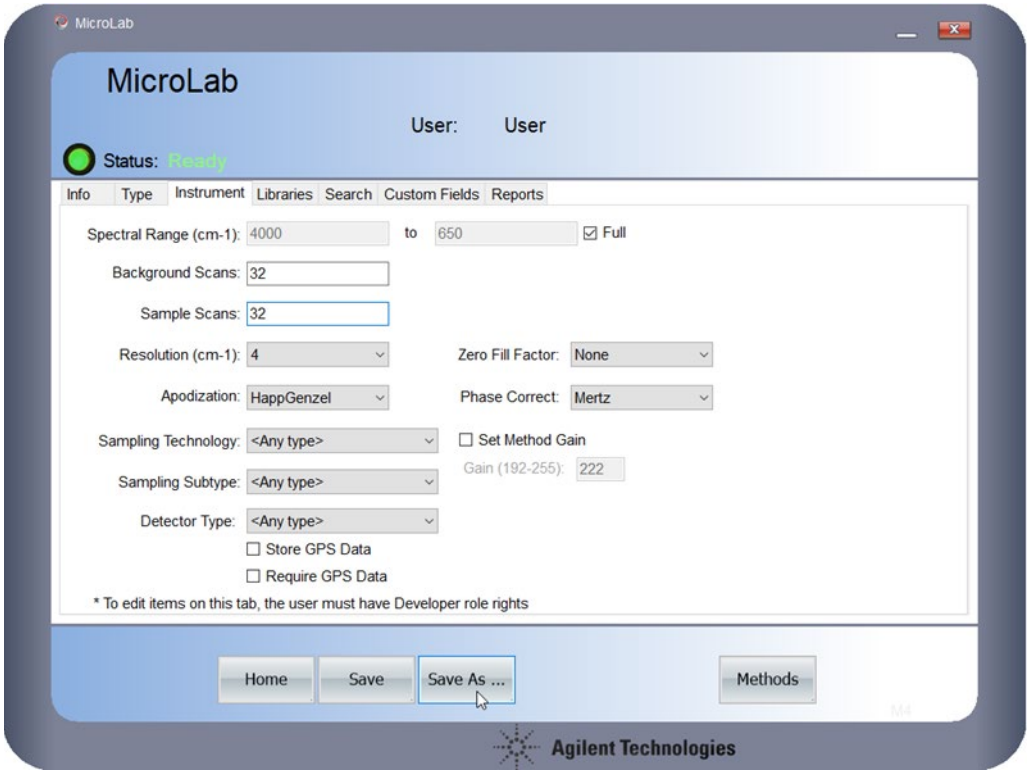


Figure 2. Instrument parameters

9 Click Save As and save the method with a descriptive file name. We used Hand_Sanitizer_data_collect for this example.

5.2 Measure the standard samples

NOTE The following instructions use ethanol as example but can equally be applied for isopropanol.

To measure the standard samples:

1 Start MicroLab PC.

2 Click Methods.

3 Select the previously created data collection only method.

4 Click Activate.

5 Click Start on the Home screen.

6 When prompted, clean the crystal.

Use an extra-low lint, non-abrasive wipe and a suitable solvent, such as acetone, ethanol or isopropyl alcohol. See the Agilent Cary 630 FTIR User's Guide for details on cleaning.

Ensure no solvent residue is present on the crystal before proceeding.

7 Click Next.

A "crystal check" and "background collect" are performed.

8 Click Next on the “Sampling Screen” when it is displayed.

9 When prompted, enter a descriptive Sample ID.

NOTE We used 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and pure ethanol standard solutions in this example and the same concentrations when repeated for the isopropanol standards.

10 Place a small volume of the standard sample onto the ATR crystal.

The liquid should cover the ATR crystal.

Ensure there is enough sample to prevent evaporation from affecting the measurement. Do not add too much sample.

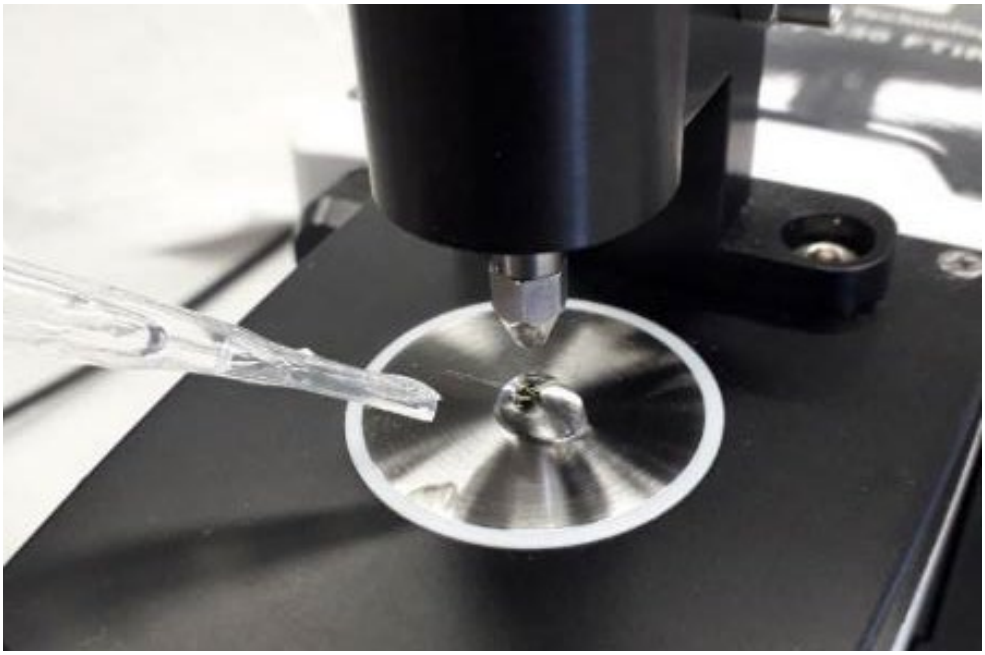


Figure 3. Sample placed on the ATR crystal

11 Click Next.

12 Clean the ATR crystal with a cloth or wipe. See the Agilent Cary 630 FTIR User’s Guide for details on cleaning.

13 Click Done.

14 Repeat Steps 6-14 until all standard samples are measured.

NOTE In order to minimize cross contamination effects, it is good practice to start with the lowest concentration standard before moving the next highest concentration standard, and so on.

15 Click Home.

5.3 Create a quantification model

1 Open MicroLab Quant.

2 Click Start New Model.

3 Select the recorded spectra of the standard samples.

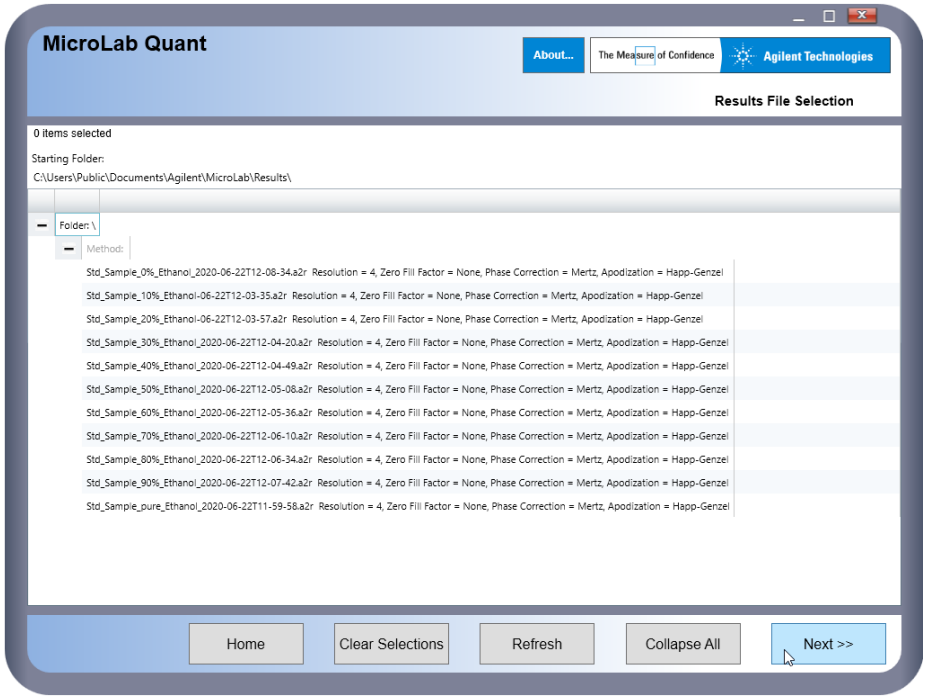


Figure 4. Selecting the previously measured standard sample spectra

4 Click **Next**.

5 Enter the actual concentration of the standard samples. The samples can appear in any order.

NOTE We used 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and pure ethanol standard solutions in this example.

6 Select **Simple Beer's Law** from the "Quant Algorithm" drop-down menu.

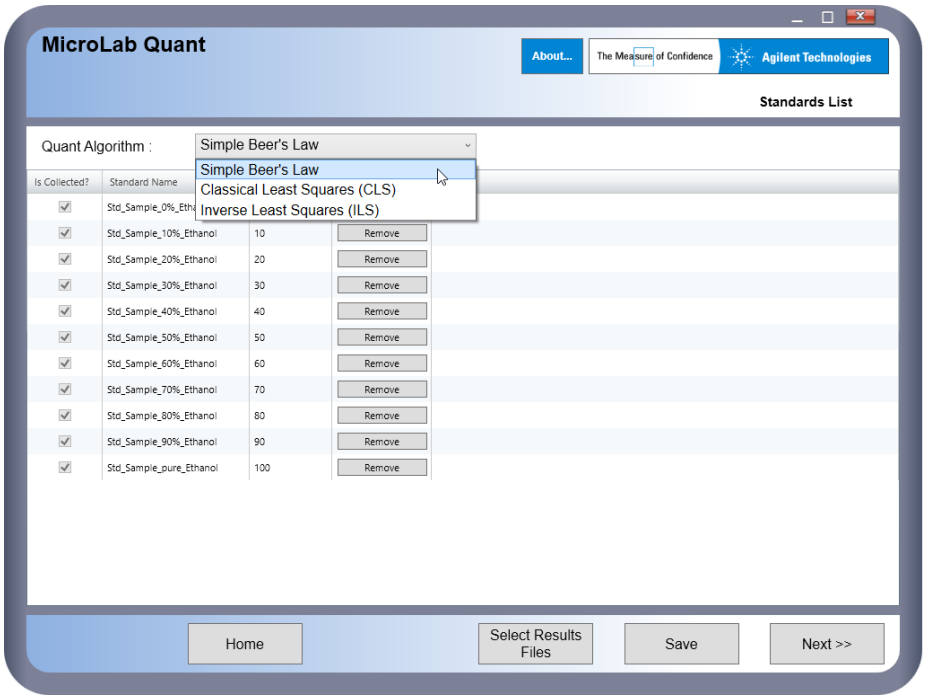


Figure 5. Selecting the quant algorithm

7 Click **Next**.

8 Zoom in the region between 1300 cm^{-1} and 800 cm^{-1}.
To do this, drag a box around the region while pressing the left mouse button.

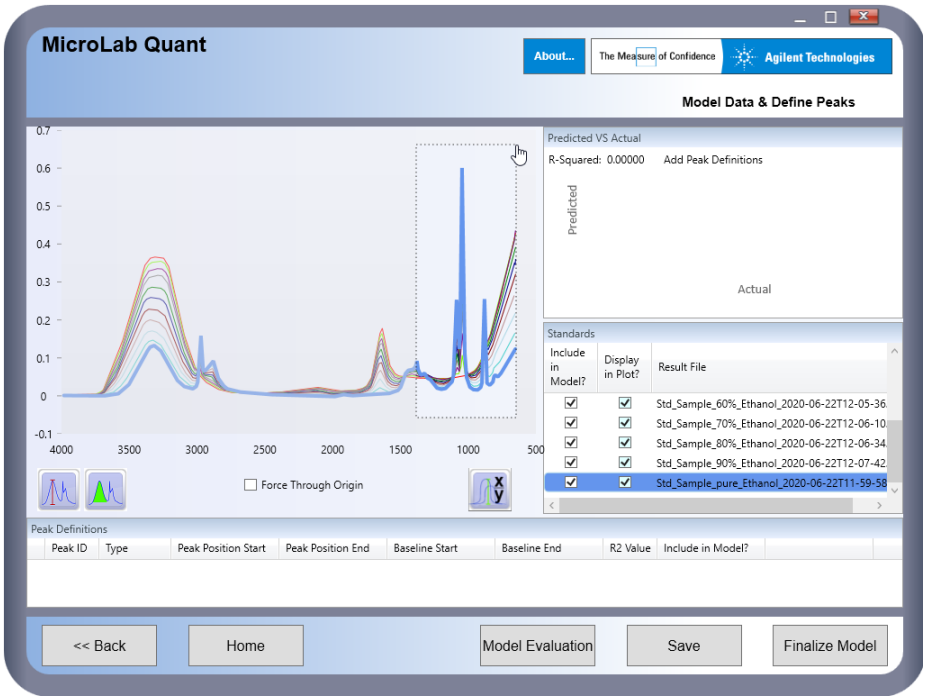


Figure 6. Zooming into the 1300 cm^{-1} and 800 cm^{-1} region

9 Drag and then release the  (**Peak Area**) icon onto the peak you want to use for quantification.

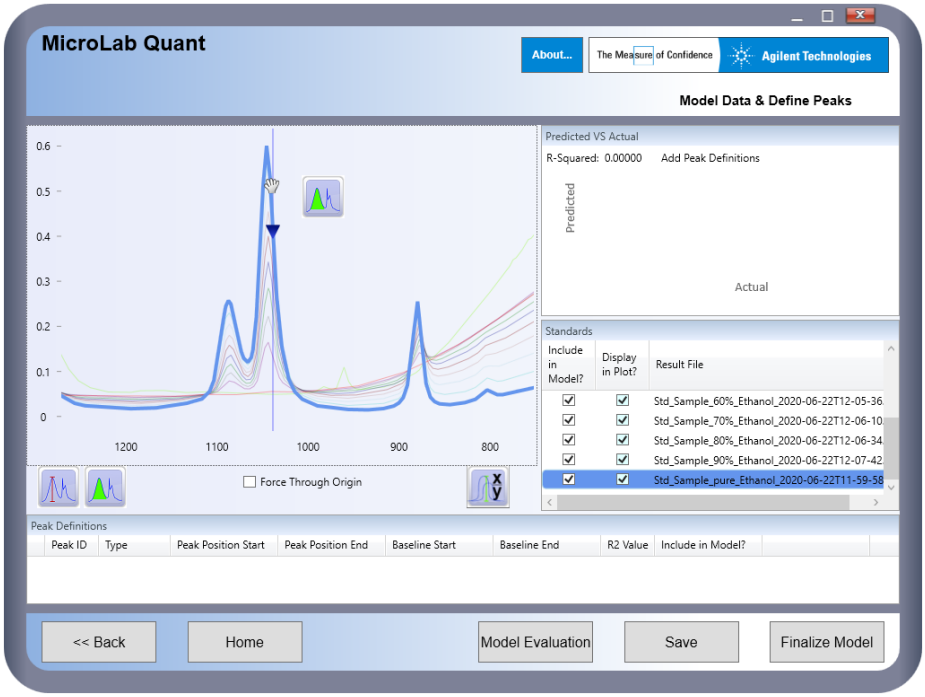


Figure 7. Drag and drop the Peak Area icon

NOTE

- For ethanol: Use the double maximum around 1080 cm^{-1} and 1045 cm^{-1}
- For isopropanol: Use the band with peaks around 1160 cm^{-1}, 1130 cm^{-1} and 1105 cm^{-1}

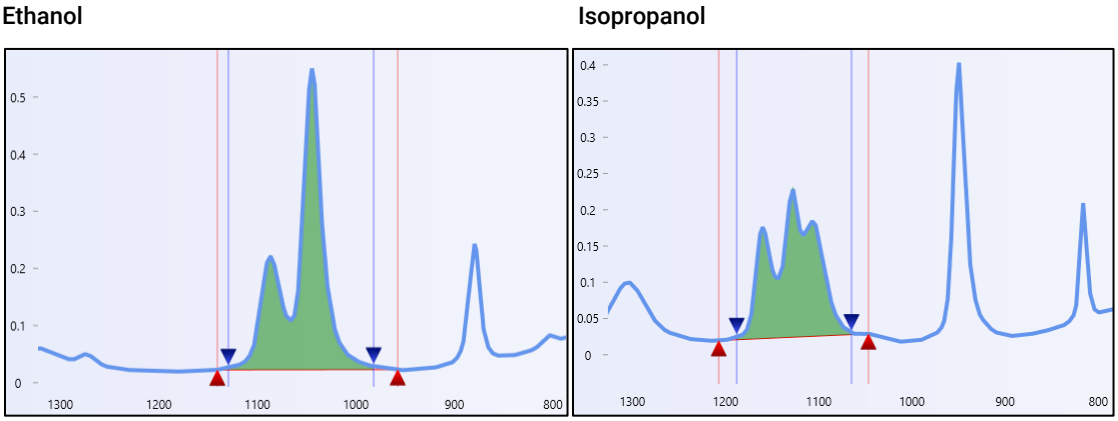


Figure 8. Peak areas for ethanol and isopropanol

10 Adjust the left and right baseline points (▲) and the left and right peak definition points (▲) to optimize the quantification model (maximize the R-Squared value). For a good calibration the R-Squared value should be around 0.99.

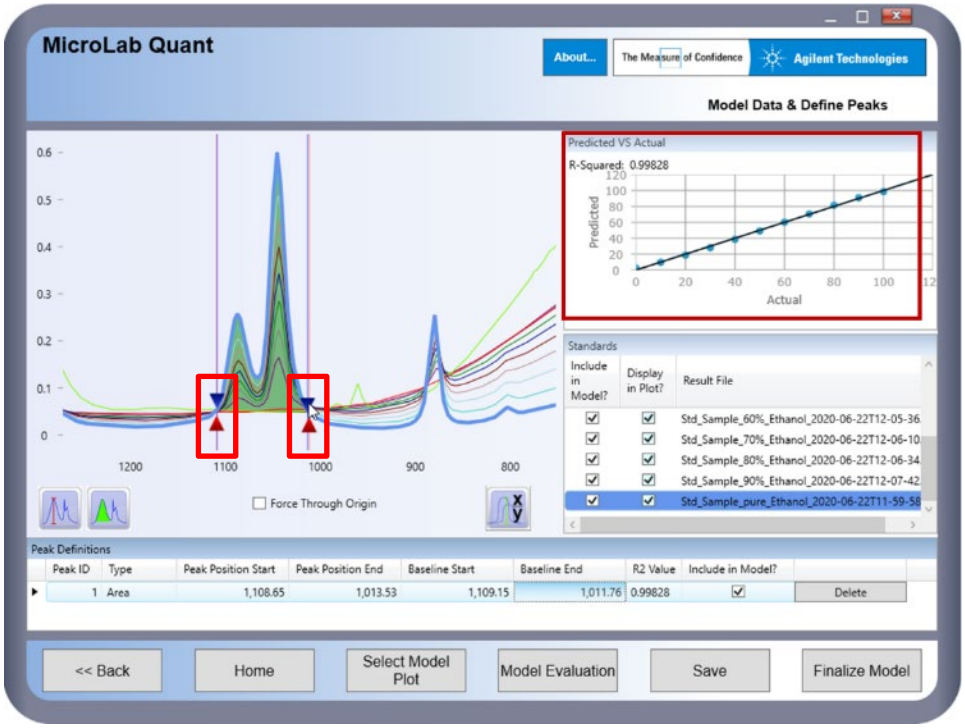


Figure 9. Peak areas definition point optimization

11 Click **Finalize Model** when the optimization is done.

12 Click **Save MLQuant Project/Model As** and then save the model with a descriptive name. In this example we used "Quant_Model_Ethanol". When we repeated the procedures for Isopropanol we used "Quant_Model_Isopropanol".

13 Close the MicroLab Quant application.

NOTE Repeat Sections 5.1, 5.2 and this section using isopropanol (or ethanol), if needed.

5.4 Create a QC method for routine analysis of ethanol in hand sanitizer

NOTE The following instructions use ethanol as example but can equally be applied for isopropanol.

To create a QC method:

1. Start MicroLab PC or MicroLab Lite.
2. Click **Methods**.
3. Select the data collection method created in Section 5.1.
4. Click **Edit**.
5. Click the **Type** tab.
6. Select **Components** from the "Method Type" drop-down menu.

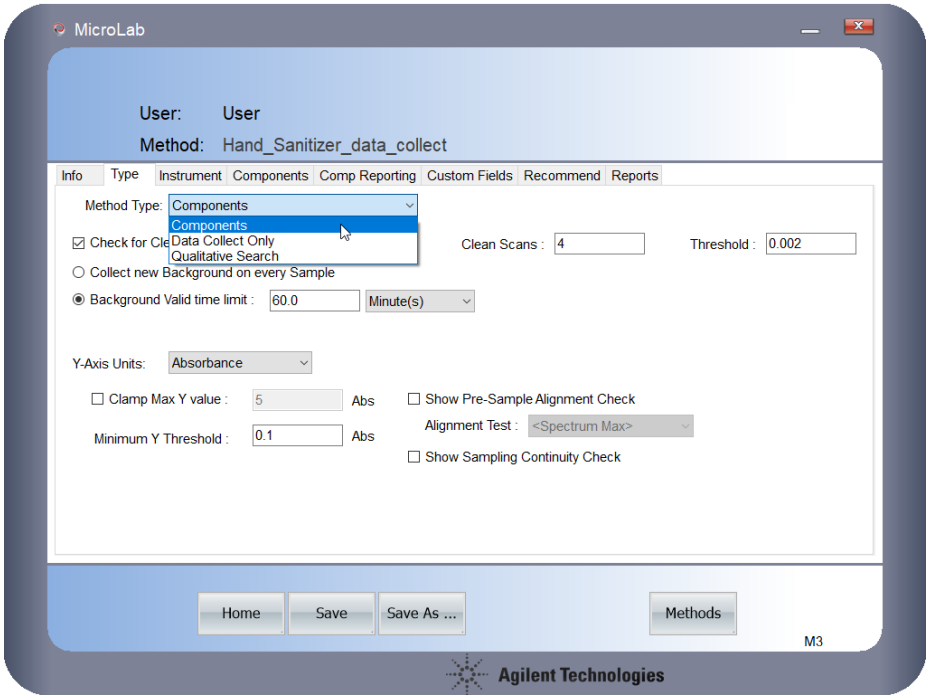


Figure 10. Selecting the method type "Components"

7. Click the **Components** tab.
8. Click **Add**.
9. Enter the component name on the "Add Component" screen. In this example we used "Ethanol Concentration". When we repeated the procedures for Isopropanol we used "Isopropanol Concentration".
10. Select **Quant Model** from the "Calculation Type" drop-down menu.

    Selecting Quant Model opens the "Select Quant Model" screen.

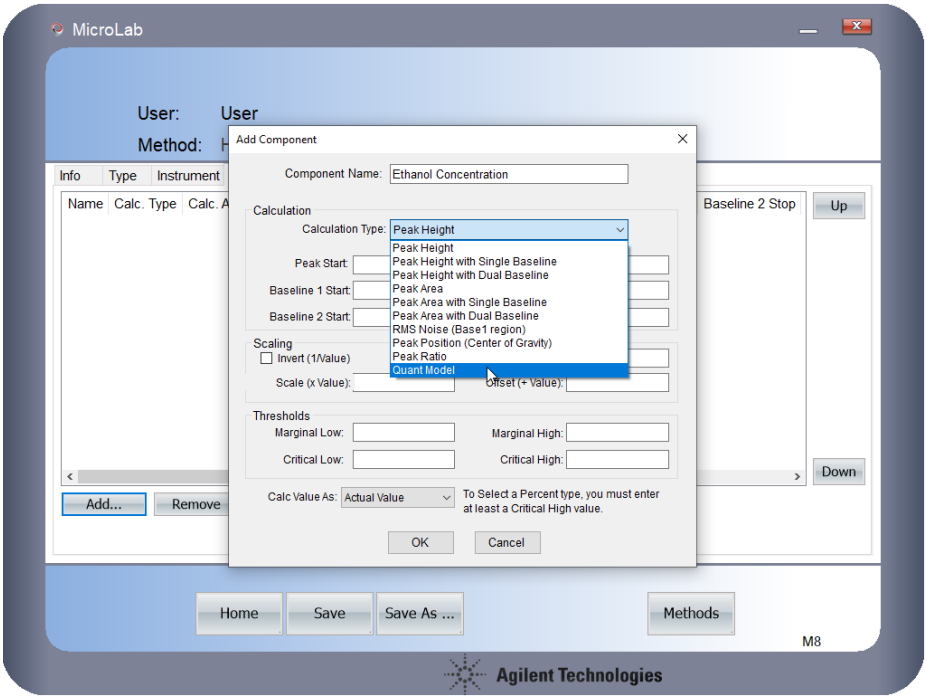


Figure 11. Selecting the calculation type "Quant Model"

11 Select the file type **(*.mqm)** from the File Name type drop-down menu to filter the list.

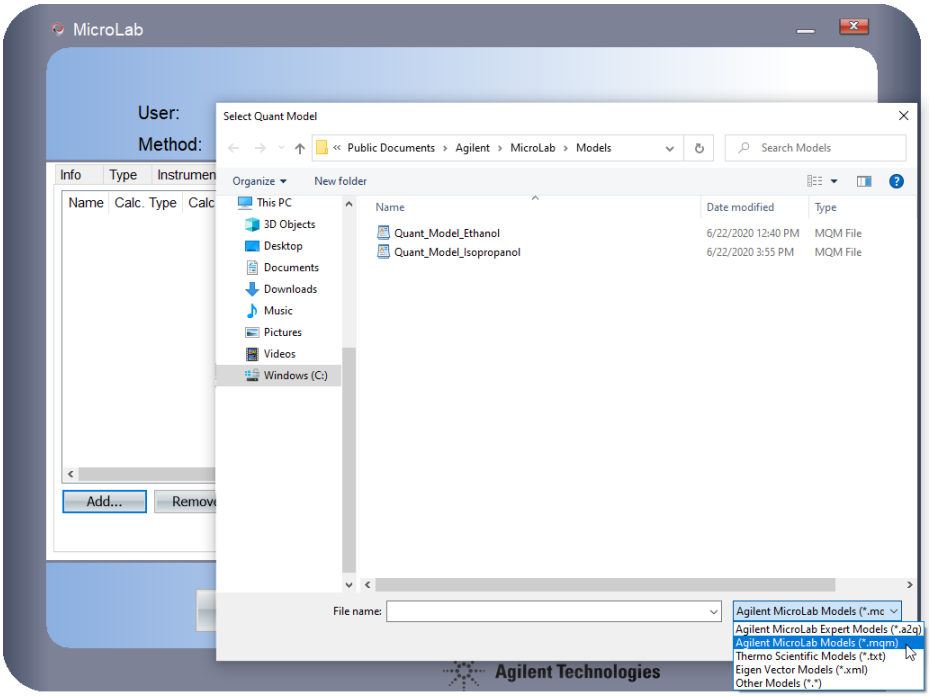


Figure 12. Selecting the Quant model

12 Select the correct Quant model from the models previously created. In this example we used "Quant_Model_Ethanol".

13 Click **Open**.

14 Set the "Decimal Digits To Report" (e.g., "1") and the "Critical Low" (= the lower acceptance limit, e.g., 60).

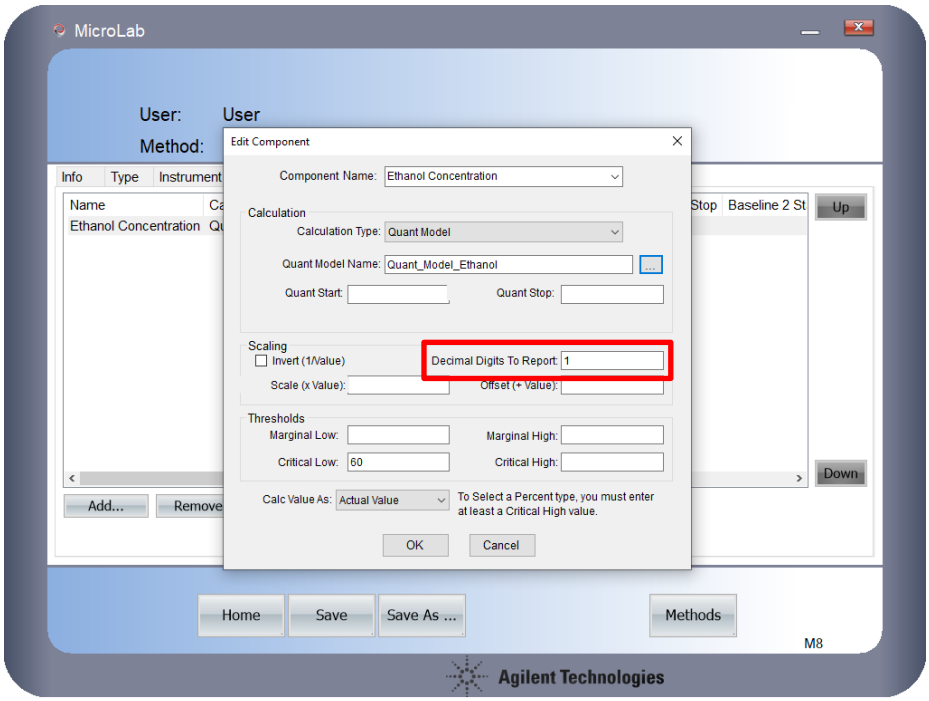


Figure 13. Setting the number of decimal digits to report

15 Click **OK**.

16 Click **Save As** and save the method with a descriptive file name. In this example we used "Hand_Sanitizer_Ethanol_Concentration". When we repeated the procedures for Isopropanol we used "Hand_Sanitizer_Isopropanol_Concentration".

NOTE Repeat this section using isopropanol, if needed.

5.5 Running the Hand Sanitizer method to analyze samples

To run the method:

1 Start MicroLab PC.

2 Click **Methods**.

3 Select the previously created routine "Hand_Sanitizer_Ethanol_Concentration" method.

4 Click **Activate**.

5 Click **Start** on the Home screen.

6 When prompted, clean the crystal. See the Cary 630 FTIR User's Guide for instructions.

7 Click **Next**.

A "crystal check" and "background collect" are performed.

8 Click **Next** on the "Sampling Screen" when it is displayed.

9 When prompted, enter a descriptive Sample ID.

10 Place a small volume of the sample onto the ATR crystal.

The liquid should cover the ATR crystal.

Ensure there is enough sample to prevent evaporation from affecting the measurement. Do not add too much sample.

11 Click **Next**.

12 Review the result.

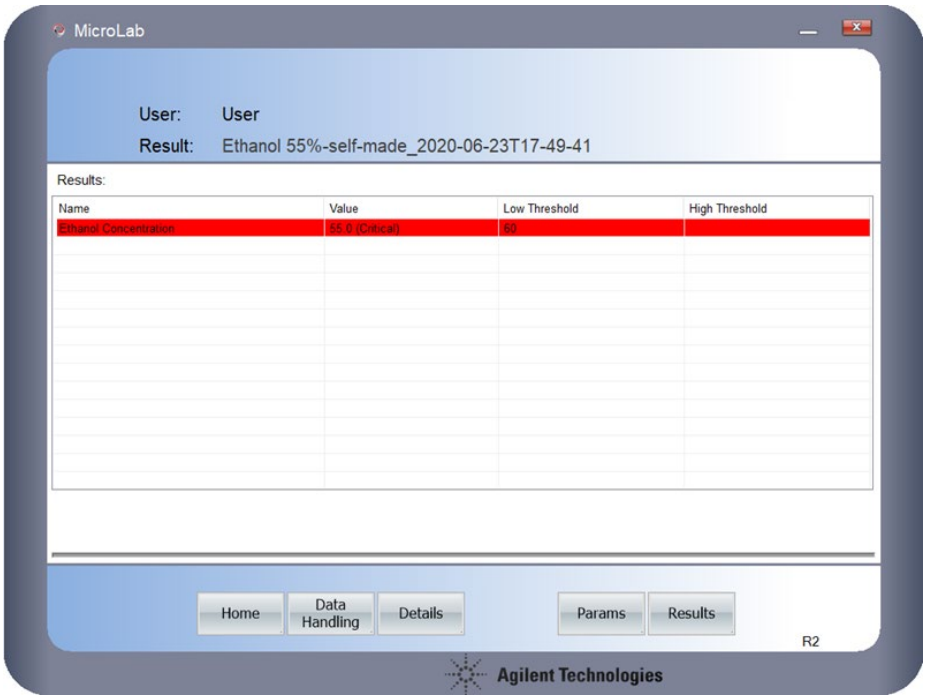


Figure 14. Example ethanol results

13 Clean the crystal. See the Cary 630 FTIR User's Guide for instructions.

14 Click **Done**.

15 Click **Next** to continue with the next sample or **Home** if you have finished the analysis.

NOTE Repeat this section using isopropanol, if needed.

6 Create One Method to Identify and Quantify Ethanol and Isopropanol in Hand Sanitizer

The methods created in Chapter 5 can be used to quantify the ethanol and isopropanol level in hand sanitizers.

The spectra below of ethanol and isopropanol show some significant spectral differences in the fingerprint region.

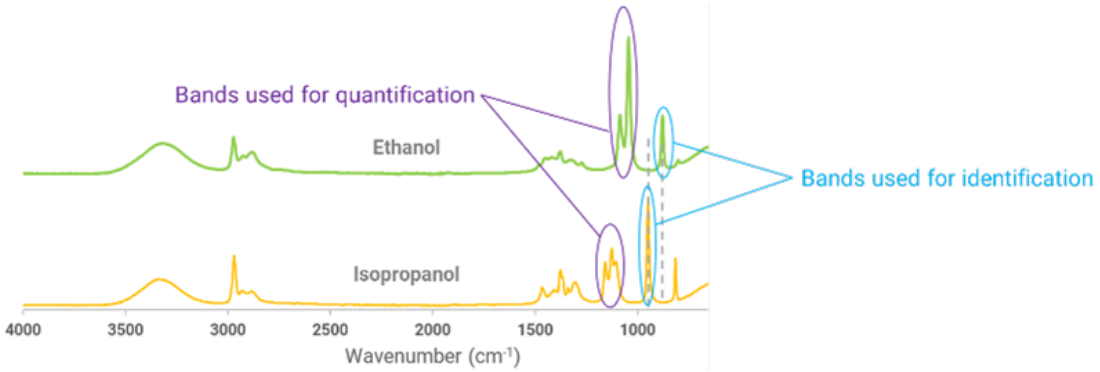


Figure 15. Ethanol and isopropanol bands used for quantification and identification

These differences can be used to distinguish between ethanol and isopropanol-based hand sanitizers. The use of another Quant model allows the analyst to create a single MicroLab method that automatically identifies the alcohol and applies the correct quantification model.

The steps involved are:

- Create a Quant model for ethanol and isopropanol differentiation
- Create one method that includes ethanol and isopropanol differentiation and quantification
  - Create a new method from the existing ethanol method
  - Add the isopropanol quantification component
  - Add the identification of ethanol and isopropanol components
  - Edit the reporting for the different analysis outcomes
- Run the method

6.1 Create a Quant model for ethanol and isopropanol differentiation

NOTE Before starting the procedure below you must have completed Sections 5.1, 5.2 and 5.3 for both ethanol and isopropanol and Section 5.4 for ethanol.

To create a Quant model that includes both alcohol spectra:

1 Start MicroLab Quant.

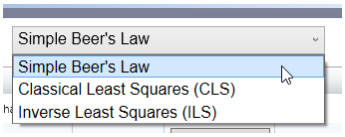
2 Click **Start New Model**.

3 Select the two spectra of the pure alcohols.

4 Click **Next**.

5 Enter “100” as the concentration of the pure isopropanol and “0” as the concentration of the pure ethanol.

6 Select **Simple Beer’s Law** as the “Quant Algorithm” from the drop-down menu.



7 Click **Next**.

8 Zoom in the region between 1300 cm^{-1} and 800 cm^{-1}.

To do this, drag a box around the region while pressing the left mouse button.

9 Click to select the isopropanol trace.

10 Drag and then release the  (**Peak Height**) icon onto the peak around 950 cm^{-1}.

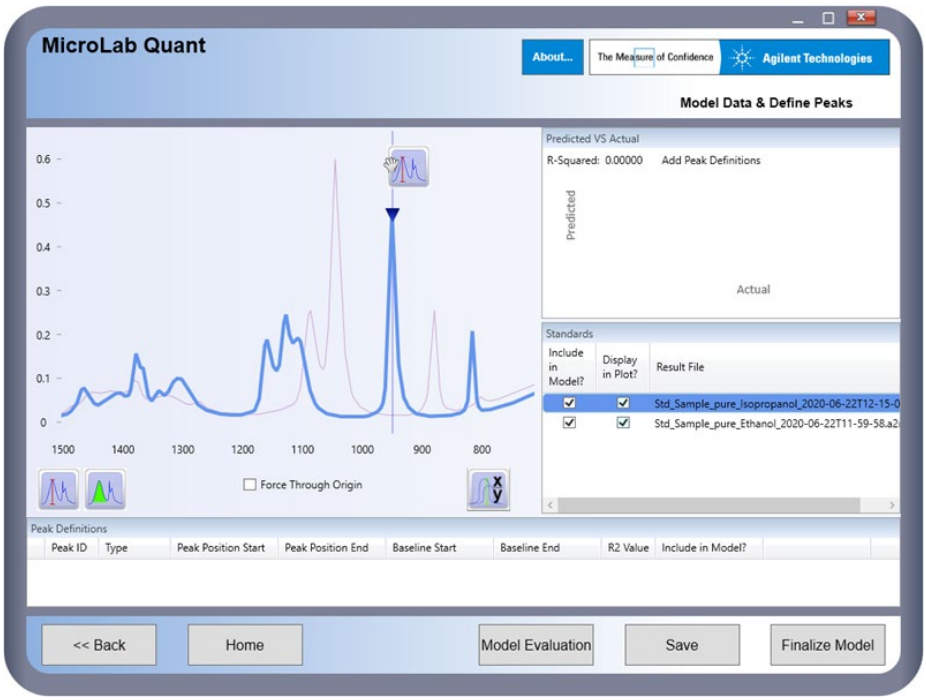


Figure 16. Isopropanol peak definition selection

11 Optimize the baseline start and end position (▲).

12 Select the ethanol trace by clicking on the trace.

13 Drag the  icon onto the peak around 879 cm^{-1}.

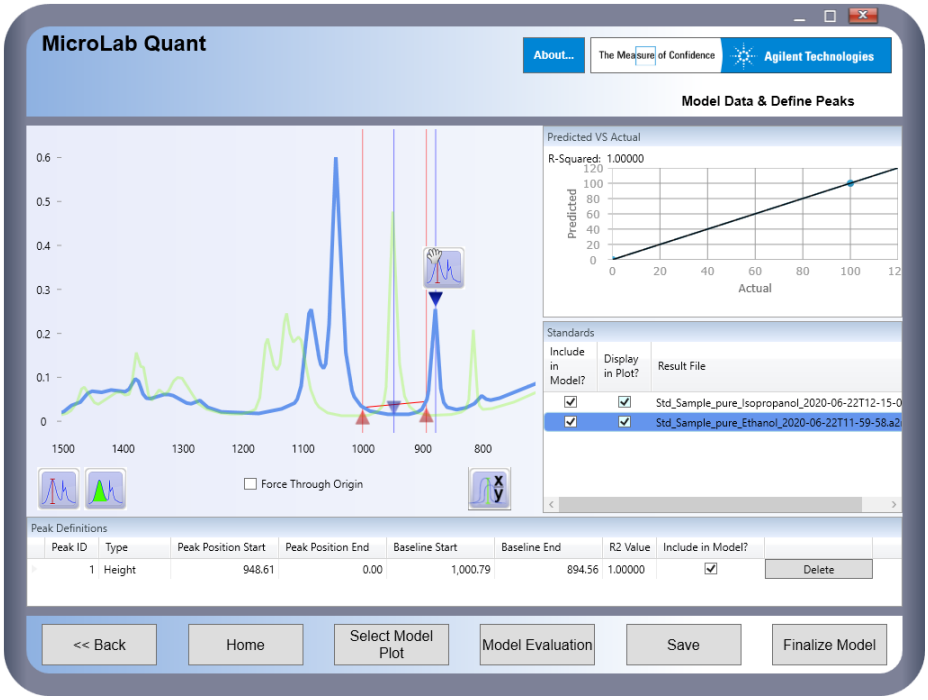


Figure 17. Ethanol peak definition selection

14 Optimize the baseline start and end position (▲).

15 Click the  (**Peak IDs to be Ratioed**) icon and select Peak ID 1 as the "Numerator" and Peak ID 2 as the "Denominator".

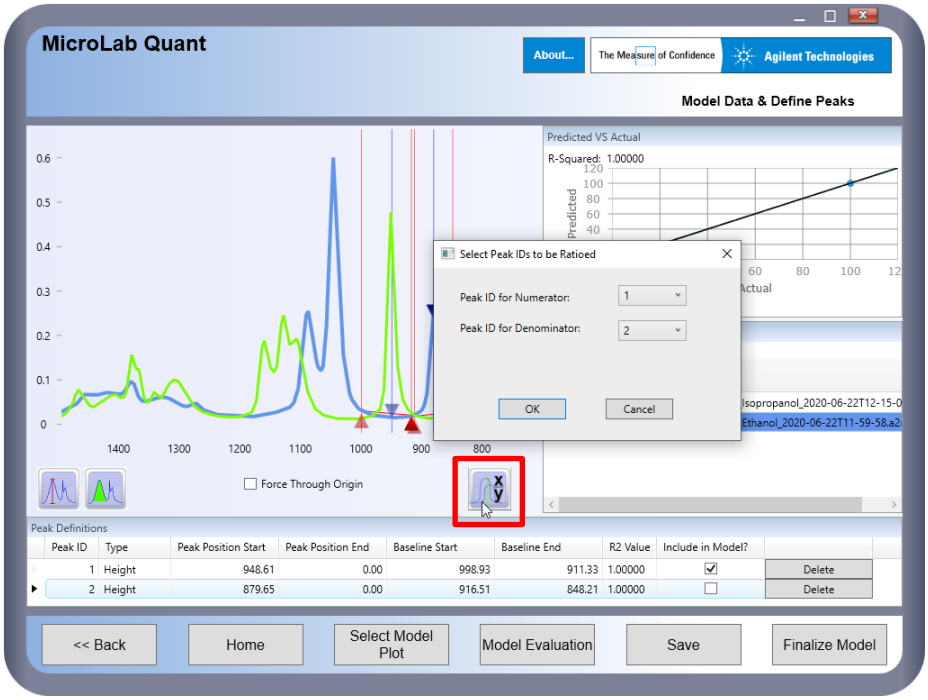


Figure 18. Selecting the Peak IDs to be ratioed

16 Click **OK**.

17 Select **Include in Model** in the "Ratio 1/2" row of the "Peak Definitions" table.

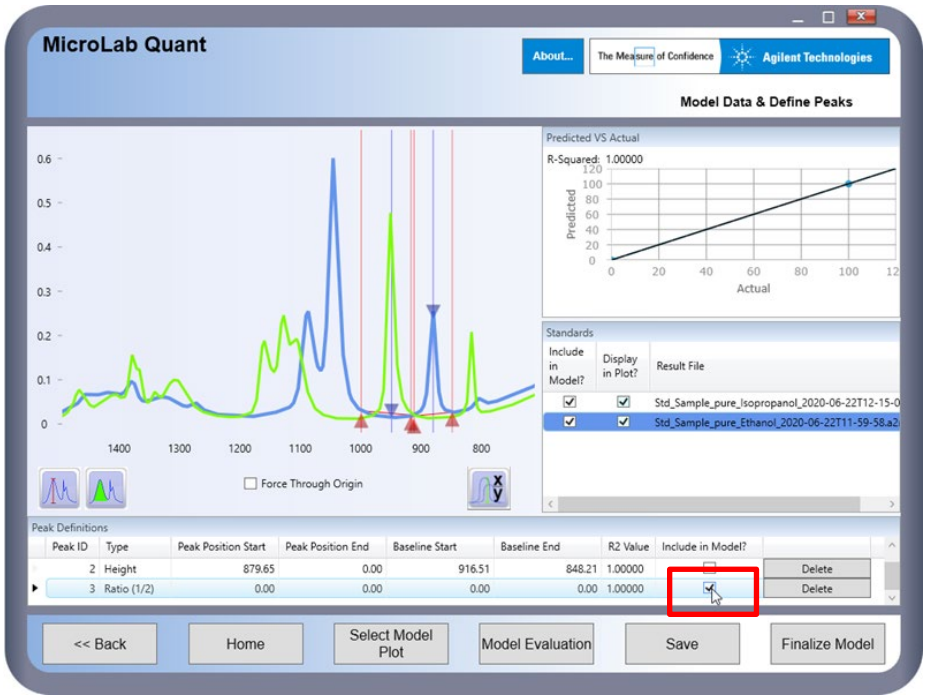


Figure 19. Including the ratio 1/2 in the model

18 Click **Model Evaluation**.

19 Click the **Independent Set** tab.

20 Click **Add Files**.

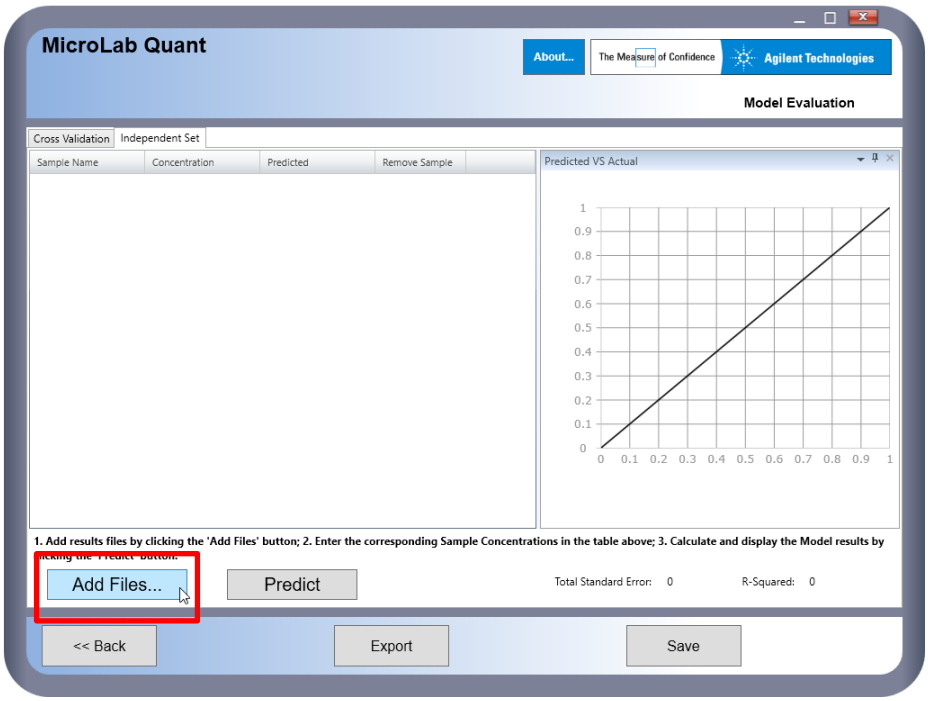


Figure 20. Adding results to the model for evaluation

21 Select all standard ethanol and isopropanol spectra.

22 Click **Open**.

23 Click **Predict**. You do not need to change the concentrations.

24 Review the values in the “Predicted” column.

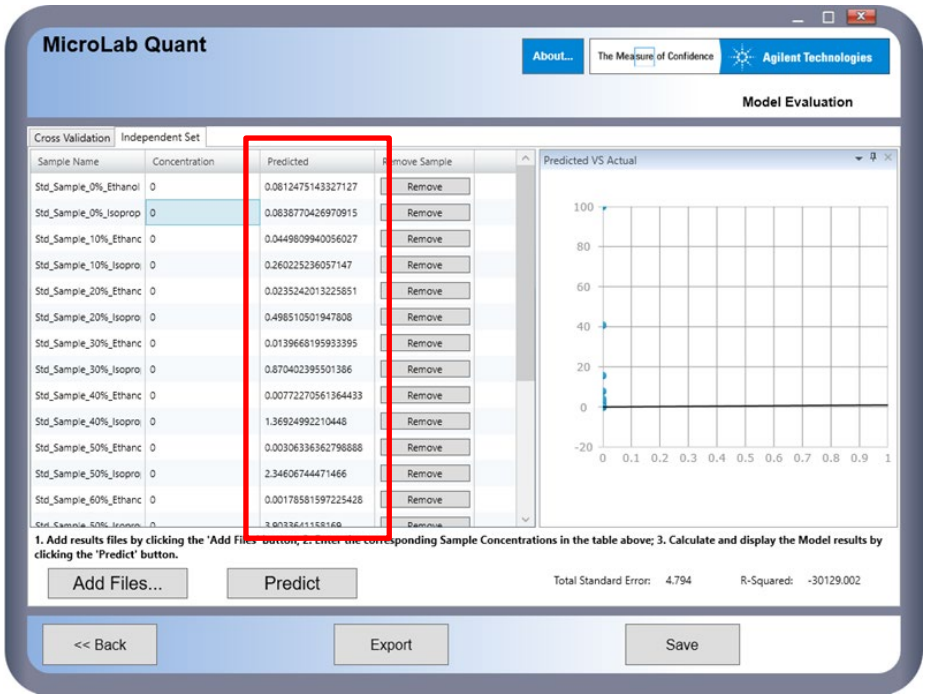


Figure 21. Predicted versus actual values

Note the highest value for any ethanol standard and the lowest value for any isopropanol standard (ignore the 0% standards). These values will be used later in this procedure.

25 Click **Back**.

26 Click **Finalize Model**.

27 Click **Save MLQuant Project/Model As** and save with a descriptive file name. In this example, the file was saved as Quant_Method_Ethanol_vs_Isopropanol.

28 Close MicroLab Quant.

6.2 Create one method for ethanol and isopropanol differentiation and quantification

The following section outlines how to create a method that identifies whether a hand sanitizer sample contains ethanol or isopropanol and automatically calculates the alcohol content based on the identified alcohol type. This method will use the Quant model developed in Section 6.1 to determine the type of alcohol and use the Quant models for ethanol and isopropanol developed in Section 5.3 to quantify the level of alcohol.

To do this you:

- Create a new method
- Add the components
- Edit the reporting for the different analysis outcomes

Create a new method

To create a new method:

1 Start MicroLab PC or MicroLab Lite.
2 Click **Methods**.
3 Select the "Hand_Sanitizer_Ethanol_Concentration " method for ethanol created in Section 5.4.
4 Click **Edit**.
5 Click **Save as** and save with a descriptive name. In this example we used "Hand_Sanitizer_Identification_Quantification". The currently open method closes.
6 Select the new method from the list.
7 Click **Edit**.

Add the components to the method

Add the isopropanol quantification component to the method

To add a new component for isopropanol quantification:

1 Click the **Components** tab.
2 Click **Add**.
3 Enter the component name "Isopropanol Concentration" on the "Add Component" screen.
4 Select **Quant Model** from the "Calculation Type" drop-down menu. See Figure 11 on Page 14.
5 Select the file type **(*.mqm)** from the File Name type drop-down menu to filter the list. See Figure 12 on Page 14.
6 Select the Quant_Model_Isopropanol previously created.
7 Click **Open**.
8 Set the "Decimal Digits To Report" (e.g. "1") and the "Critical Low" (= the lower acceptance limit, e.g. 60).

Add the identification components to the method

Add the ethanol identification component

1 Click the **Components** tab.
2 Click **Add**.
3 Enter the component name 'Sample contains "Ethanol"'.
4 Select **Quant Model** from the "Calculation Type" drop-down menu.
5 Select the file type **(*.mqm)** from the File Name type drop-down menu to filter the list.
6 Select the Quant model Quant_Method_Ethanol_vs_Isopropanol previously created in Section 6.1.
7 Enter 2 in the "Decimal Digits To Report" field.
8 Find the highest value of the ethanol standards in the Predicted column in the Quant model and note it down. See Figure 21 and Step 24 in the section "Create a Quant model for ethanol and isopropanol differentiation" for more information.
9 Find the the lowest value of the isopropanol standards in the Predicted column in the Quant model and note it down. Ignore the 0% standards.
10 Choose a value sitting evenly between these two values.
11 Enter that value in the "Critical High" field of the Edit Component screen. We chose 0.1 for this example. See Figure 22.
12 Click **OK**.

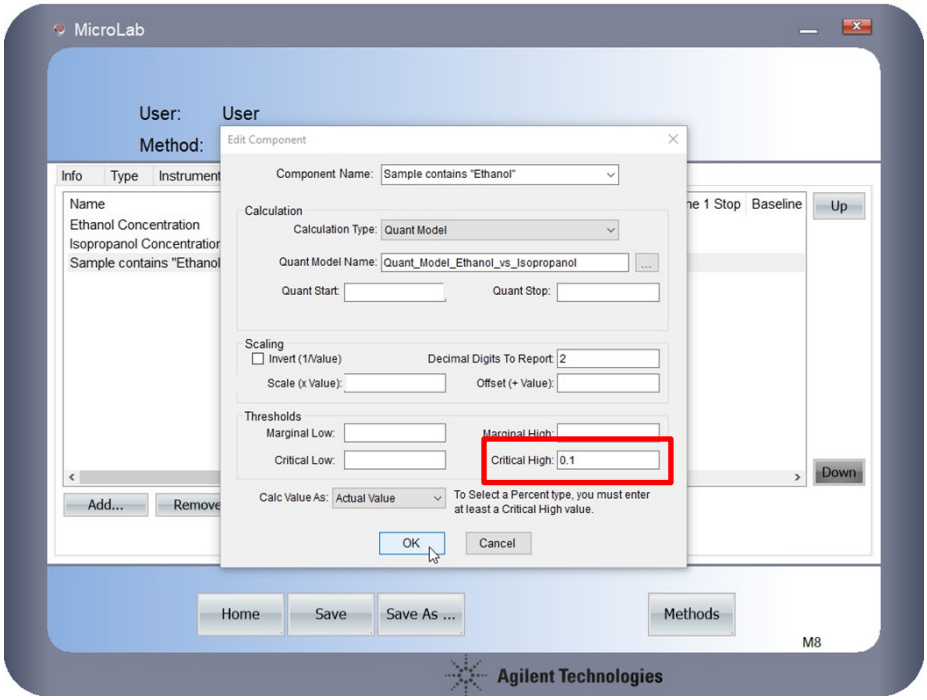


Figure 22. Entering the critical high value for ethanol indentification

Add the isopropanol identification component

1. Click the **Components** tab.
2. Click **Add**.
3. Enter the component name 'Sample contains "Isopropanol"'.
4. Select **Quant Model** from the "Calculation Type" drop-down menu.
5. Select the file type **(*.mqm)** from the File Name type drop-down menu to filter the list. See Figure 12 on Page 14.
6. Select the Quant model previously created in Section 6.1.
7. Enter 2 in the "Decimal Digits To Report" field.
8. Enter the same value entered in Step 10 in the "Add the ethanol identification component" section into the "Critical Low" field. We chose 0.1 for this example. See Figure 23 on Page 24.
9. Click **OK**.

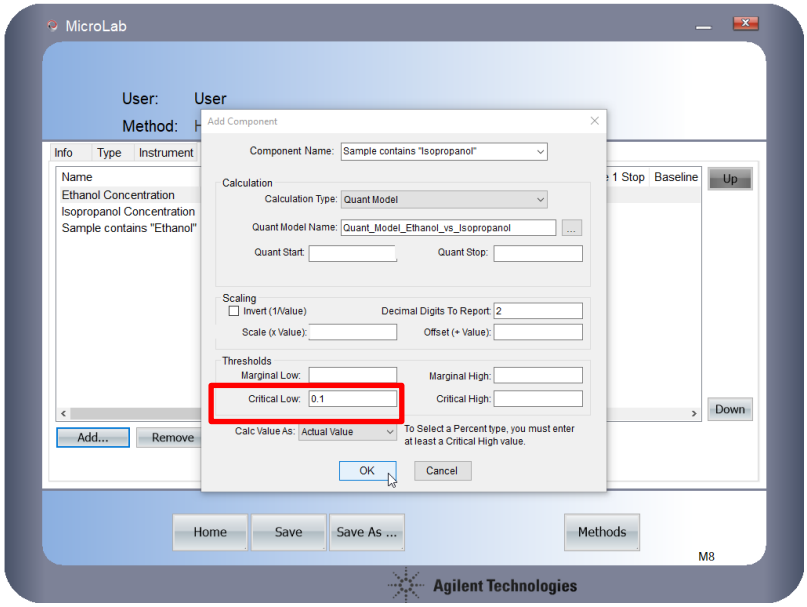


Figure 23. Isopropanol identification component parameters

Edit the reporting for the different analysis outcomes

In the following section logic rules are used to ensure the ethanol concentration is only reported when the “Sample contains “Ethanol” component result is below the “Critical High” value. The same approach is used for the isopropanol concentration reporting.

Edit the “Ethanol Concentration” reporting

1 Click the **Comp Reporting** tab.

2 Select the component named “Ethanol Concentration”

3 Click **Edit**.

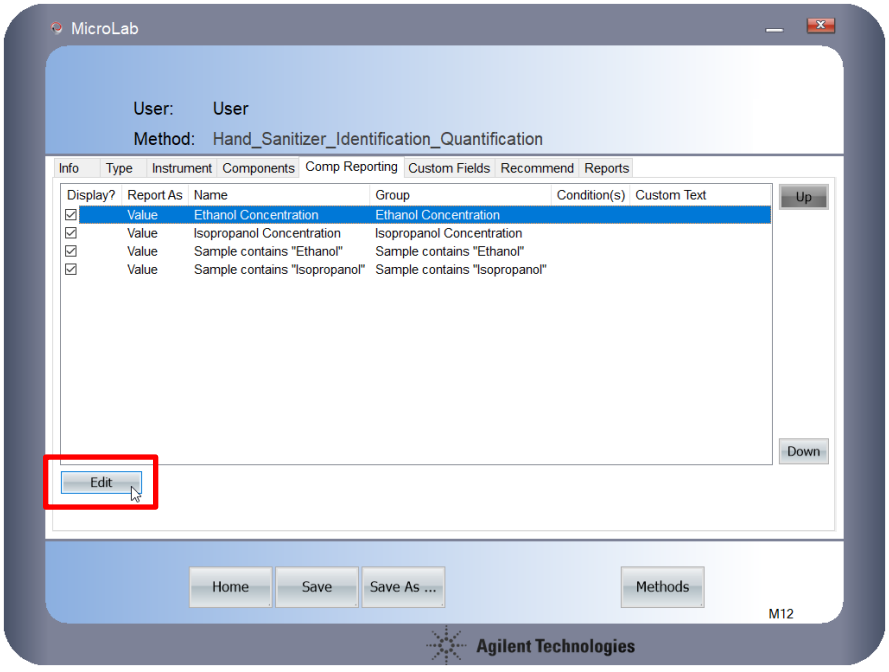


Figure 24. Comp reporting editing for Ethanol Concentration

4 Select **Sample contains "Ethanol"** in the "Component / Diagnostic" drop down menu on the "Edit Component Reporting Condition" screen.

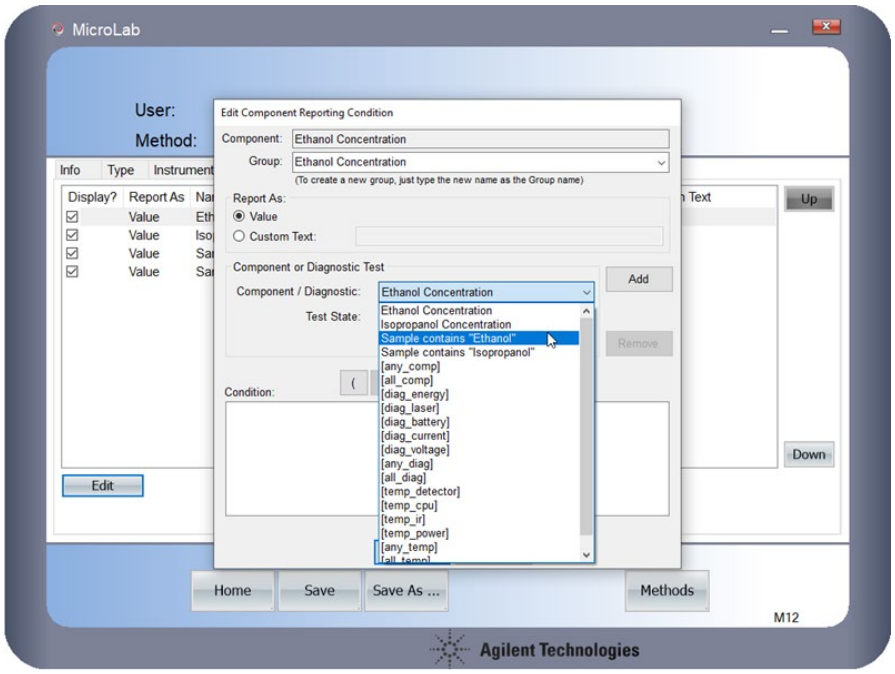


Figure 25. Selecting the Component/Diagnostic option

5 Click **Add**.

The "Condition" phrase "Sample contains "Ethanol" is Good" is created.

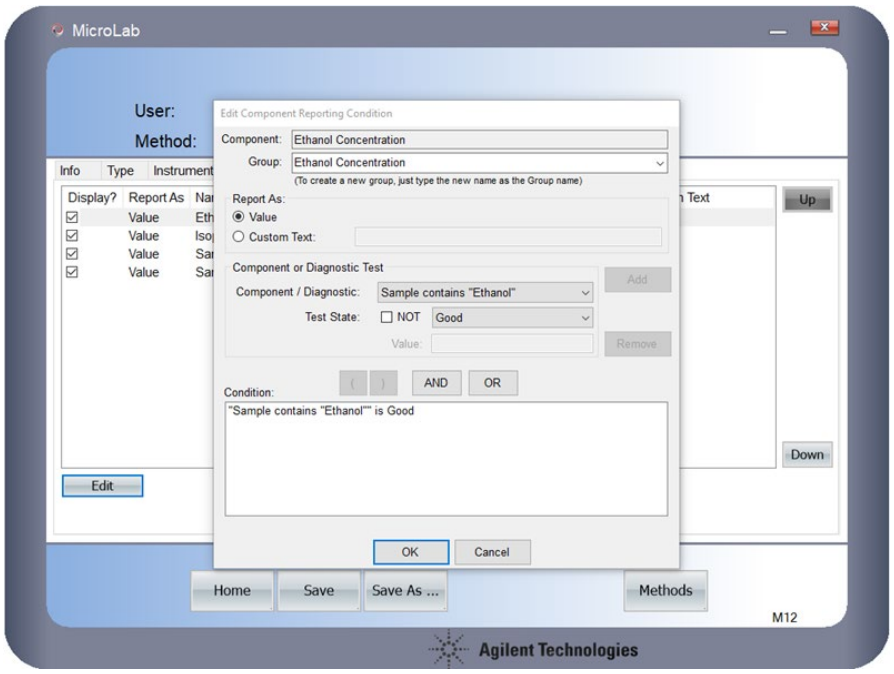


Figure 26. Sample contains "Ethanol" is Good message created

6 Click **OK**.

Edit the “Isopropanol Concentration” reporting

1 Select the component **Isopropanol Concentration**.

2 Click **Edit**.

3 Select **Sample contains “Isopropanol”** in the “Component / Diagnostic” drop down menu on the “Edit Component Reporting Condition” screen.

4 Click **Add**.

5 Click **OK**.

Edit the “Ethanol Identification” reporting

1 Select the component **Sample contains “Ethanol”**.

2 Click **Edit**.

3 Enter “Alcohol:” in the “Group” field

4 Enter “The sample contains ETHANOL” into the “Custom Text” field.

5 Select **Sample contains “Ethanol”** in the “Component/Diagnostic” drop-down menu.

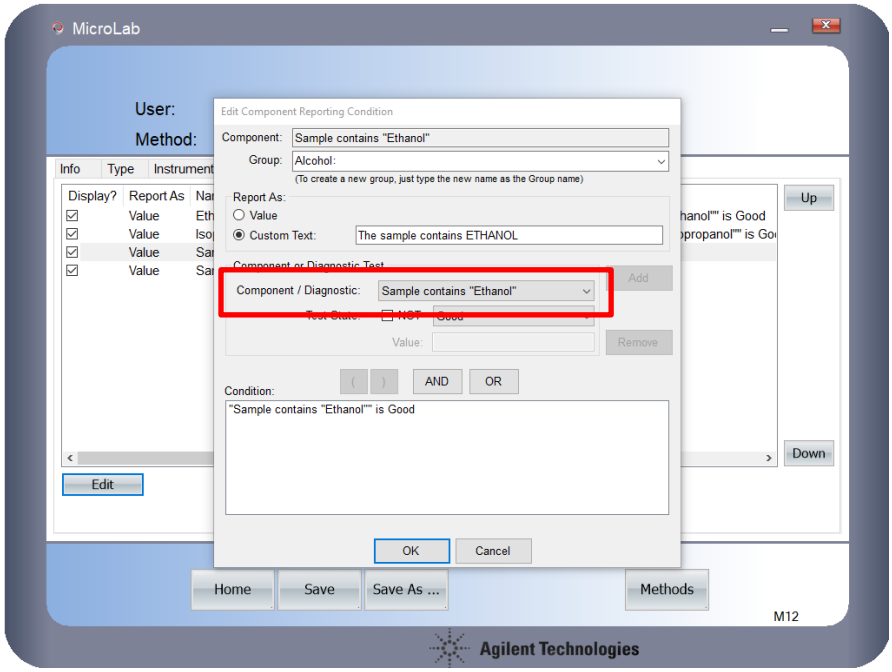


Figure 27. Editing the ethanol identification reporting parameters

6 Click **Add**.

7 Click **OK**.

Edit the “Isopropanol Identification” reporting

1 Select the component **Sample contains “Isopropanol”**.

2 Click **Edit**.

3 Enter “Alcohol:” in the “Group” field

4 Enter “The sample contains ISOPROPANOL” into the “Custom Text” field.

5 Select **Sample contains “Isopropanol”** in the “Component/Diagnostic” drop-down menu.

6 Click **Add**.

7 Click **OK**.

8 On the “Comp Reporting” tab ensure that the tick box in the “Display?” column is active for all components. See Figure 28.

9 Click **Save**.

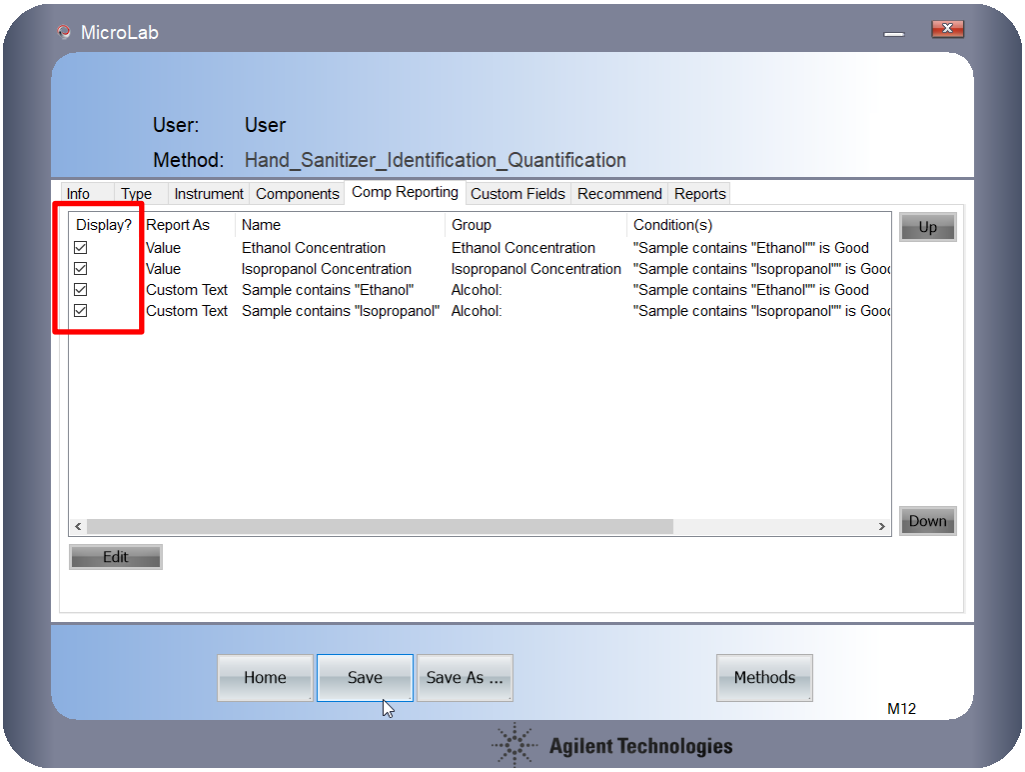


Figure 28. Editing the isopropanol identification reporting parameters

6.3 Running the Method

To start a new analysis using the method:

1 Start MicroLab PC

2 Click **Methods**.

3 Select the method created just created for ethanol and isopropanol differentiation and quantification. In this example we use “Hand_Sanitizer_Identification_Quantification”.

4 Click **Activate**.

5 Click **Start**.

6 Follow the instruction on the screen

The method identifies the type and level of alcohol present in the sample.

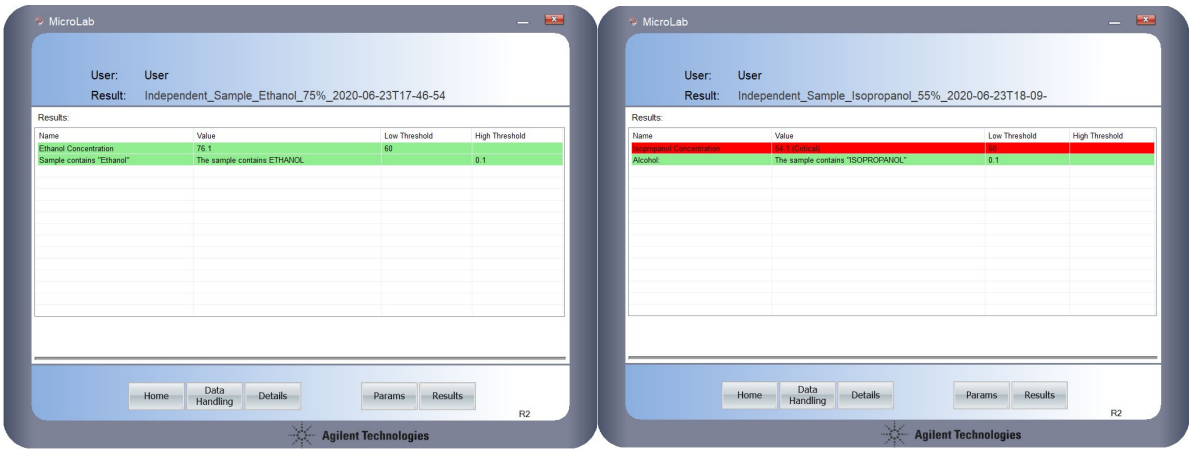


Figure 29. Example results

In This Document

The document describes the following:

- Scope
- Safety
- Equipment and Materials
- Solution and Instrument Preparation
- Preparation
- Method Development
- Create One Method to Identify and Quantify Ethanol and Isopropanol in Hand Sanitizer

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