# How to use FTIR to ensure the safety and efficacy of alcohol-based hand sanitizers: from initial research to production

Regular handwashing is incredibly important to avoid getting sick and spreading germs to others. In most situations, washing hands with soap and water is the best way to kill microorganisms, however in 2002, the Centers for Disease Control and Prevention (CDC) revised the guidelines for hand hygiene to recommend alcohol-based hand sanitizers for public and health care personnel, to reduce the transmission of pathogenic microorganisms.[1]

Most alcohol-based hand sanitizers contain either isopropanol, n-propanol or ethanol (or a combination of two of these alcohols). This alcohol content can denature proteins and therefore exhibits a broad-spectrum of antimicrobial activity, however, the efficacy is dependent on the concentration of alcohol present in the solution. Solutions containing 60-85% ethanol and/or 60-70% isopropanol show the best antimicrobial efficacy, whereas, those with less than 60% alcohol may only reduce the growth of germs instead of killing them.[2]

As a result of the coronavirus (COVID-19) pandemic, the Food and Drug Administration (FDA) issued guidelines that temporarily allowed the preparation of alcohol-based hand sanitizers by entities other than registered drug manufacturers.[3] Yet, there are challenges and concerns to consider regarding the formulations and preparation protocols, therefore, proper quality assurance and control measures are necessary to ensure the efficacy and safety of hand sanitizer products.

The research and development (R&D) stage is important for product development; intensive research is required by a R&D team as they search for the most suitable product for a specific application. By contrast, the production phase involves routine analysis to ensure that the product meets the requirements for final use. The starting materials used to produce hand sanitizers include: alcohol (active ingredient), hydrogen peroxide (disinfectant), glycerol (humectant), and sterile, distilled water, and the quality control of these ingredients is essential for safety.[5] Although alcohol concentration can be measured with an alcoholmeter, this method cannot detect small contaminations, such as methanol. Instead, detection can be rapidly performed using Fourier Transform Infrared (FTIR) spectroscopy to confirm that the ingredients meet the specifications. The final product must be carefully analyzed to check that it contains the correct alcohol concentration and is absent from chemical and microbiological contaminants before it is sent to the market.[6]

FTIR spectroscopy is an important tool used in various aspects of the process, from confirming the chemical composition of starting materials and verifying the purity of intermediate steps, to meeting regulatory requirements and verifying the final product. Thermo Scientific™ offers facile solutions to meet the analytical requirements from research to final production. This guide will provide an overview of FTIR and the different techniques used to ensure the efficacy and safety of alcohol-based hand sanitizers during each step of the workflow.

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## What is Fourier Transform Infrared (FTIR) spectroscopy?

FTIR spectroscopy is one of the most widespread analytical techniques used in academia and industry to identify and quantify diverse sample components. FTIR relies on the vibration modes of the atoms of a molecule.[7] When infrared radiation (IR) passes through a sample, a portion of it is absorbed at specific frequencies which are directly related to the atom-to-atom vibrational bond energies in each molecule. The portion of the radiation that is transmitted at the detector, results in an absorption spectrum showing the peaks where specific bond vibrations occur. Different molecules will show different peak collections, depending on their structure. Thus, the IR spectrum represents a molecular fingerprint used to identify and distinguish most substances.[5,6]

FTIR is a non-destructive and user-friendly technique, known for its high sensitivity, selectivity, and temporal resolution. Moreover, many sampling techniques are available, opening up its application to a wide range of samples, including: liquids, solutions, solids, powders, pastes, films, fibers, and gases.[7] The relatively low cost of FTIR instruments makes the technique accessible to most research centers and companies, where it is routinely used for compound identification, the determination of substances in a mixture, and process monitoring. As a result, FTIR spectroscopy is considered a valuable technique in the pharmaceutical, polymer, chemical, petrochemical, food, and catalyst industries, among others.

**Additional resources can be found at the Spectroscopy Academy which contains helpful overviews, tutorials, videos and application notes.**

## Research

Before any hand sanitizers reach the public domain, they must go through intense research and development (R&D). Research laboratories study the efficacy of different hand sanitizer formulations against target bacteria, fungi and viruses.[8,9] Additionally, they must ensure that the formulations are safe for use. During development, several tests are conducted to:

- Test the safety and efficacy of different formulations against pathogens
- Assess the purity and concentration of the starting and final products
- Ensure the final product meets the specifications
- Confirm the absence of microbiological contamination

Thermo Scientific offers a large range of instrumentation that can be used during R&D. The Thermo Scientific™ Nicolet™ iS50 FTIR spectrometer provides a highly flexible all-in-one workstation for material analysis, with purpose-built accessories and integrated software (Figure 1). The Nicolet iS50 FTIR spectrometer can be enhanced to a fully automated multi-spectral range system that can acquire spectra from far-infrared to visible, using novel attenuated total reflectance (ATR), Raman, and near-infrared (NIR) modules. Additionally, it is equipped with a built-in ATR accessory and the Thermo Scientific™ OMNIC™ Software platform, which provides a simple solution for meeting the analytical requirements.

**Figure 1. Thermo Scientific™ Nicolet™ iS50 FTIR spectrometer**

QCheck spectral correlation tool, a function of the Thermo Scientific™ OMNIC™ Paradigm software, is ideal for the verification of incoming materials, in-process materials, or finished products. Figure 2 shows the Thermo Scientific™ Nicolet™ iS50 FTIR spectrometer spectrum of denatured, industrial-grade ethanol compared against the spectrum of the non-denatured, reagent grade ethanol. The denatured ethanol spectrum shows a peak at 950 $cm^{-1}$ that corresponds to isopropanol. The QCheck tool reveals that the level of isopropanol impurity is higher than the predefined threshold. This result was achieved without constructing libraries or complex spectral manipulations which saves time and resources. Without the QCheck tool, the end-user would have to collect reference spectra

of all possible contaminants, construct their own library, and compare the sample spectrum with all the reference spectra. These procedures are time-consuming and inefficient.

QC Compare Search is another function of the OMNIC software which is useful in hand sanitizer R&D. This tool is a spectral classification method that determines the best match of a sample from a reference spectrum. It also indicates how similar the unknown sample spectrum is to the chosen standard (that is the *match value*). The WHO recommends that only United States Pharmacopeia (USP) or Food Chemical Codex (*food grade*) glycerol should be used in any formulations.[5] Figure 3 presents the results of the incoming inspection of two types of glycerol using the QC Compare function of OMNIC. The QC Compare algorithm allows setting a *critical match value*, which is the threshold that the sample similarity should overcome. In this case, with a critical match value set at 90, one glycerol passed the required specifications and, therefore, was used for hand sanitizer production, while the other sample failed the test and could not be used.

In addition to starting material analysis, FTIR can be used for the quantitative determination of the alcohol fraction in hand sanitizers. The Thermo Scientific™ TQ Analyst™ Software is ideal for this task since it has an intuitive approach to sample identification, verification, and qualitative and quantitative analysis. For example, a series of FTIR measurements of ethanol/water standards with ethanol ranging from 25-99% (v/v) were carried out to determine the concentration of commercial samples (results in Figure 4). The area of the characteristic peak of ethanol at 878 $cm^{-1}$ was used to build a calibration curve using Lambert-Beer's Law in Thermo Scientific™ TQ Analyst™ Pro Edition software. The calibration curve presents outstanding linearity with a correlation coefficient higher than 0.99. This linearity is

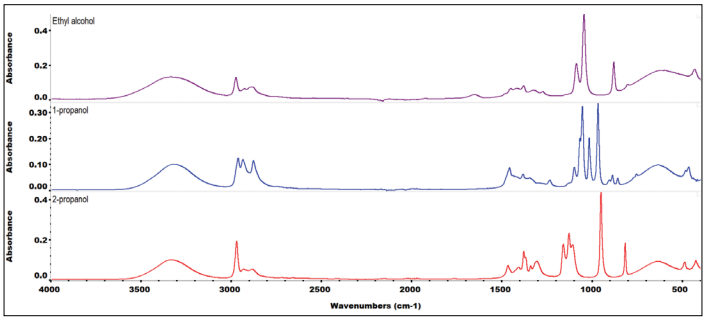



**Figure 2. ATR FTIR spectra of three alcohols commonly used in alcohol-based hand sanitizers: ethanol, 2-propanol and 1-propanol. Each spectrum is 16 scans co-added at a spectral resolution of 4 $cm^{-1}$.**

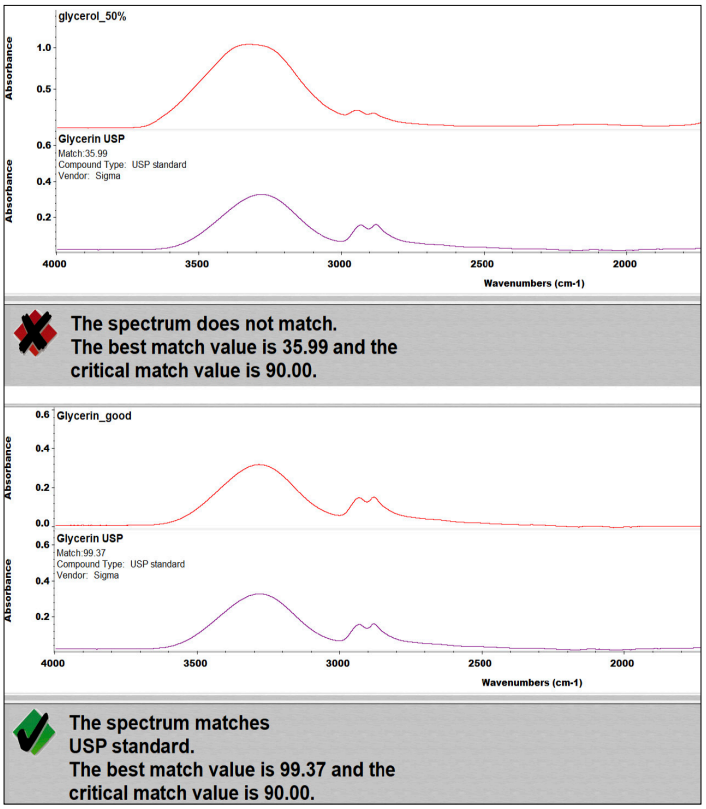


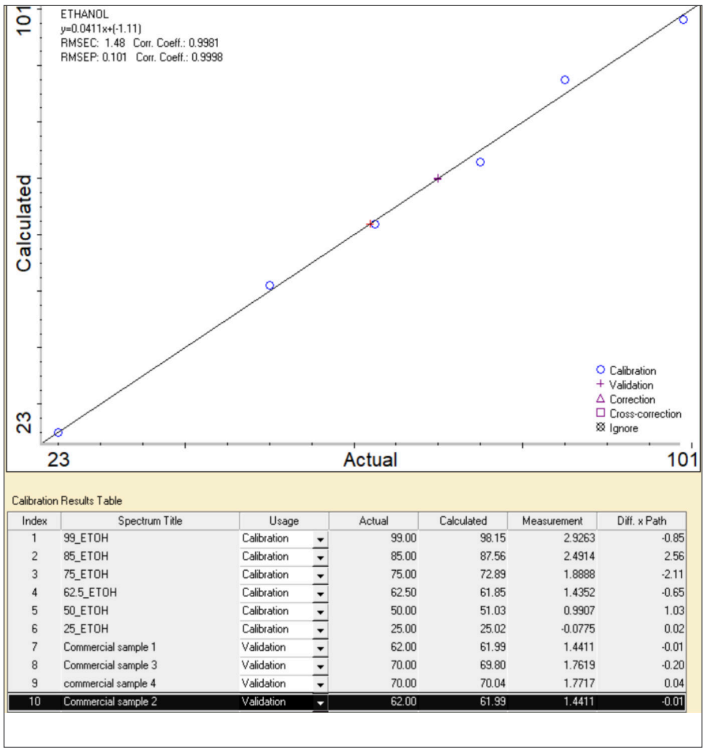

**Figure 3. QC Compare results of two types of glycerol.**

very important because it defines the range used to obtain accurate and precise results. The calibration curve can be used to determine the ethanol percentage in samples with high accuracy. Here, four commercial ethanol-based hand sanitizers were tested. The predicted ethanol concentration was very similar to the label (with an error of less than 0.3%), which demonstrates the quantitative capability of the TQ Analyst Pro Edition software.

## Production QA/QC

Quality assurance (QA) and quality control (QC) are important for ensuring that customers receive products that meet specific standards and regulations. During QA/QC analysis for hand sanitizer production, it is crucial that rapid and simple methods exist for identifying sample components so that critical decisions can be made quickly.

The Nicolet iS50 FTIR spectrometer discussed previously is too advanced for routine QA/QC analysis. Therefore, the Thermo Scientific™ Nicolet™ Summit PRO FTIR spectrometer is preferred for QA/QC laboratories as it offers quick *pass/fail* results by reducing the measurements steps. The compactly designed Nicolet Summit FTIR spectrometer




Calibration Results Table

| Index | Spectrum Title | Usage | Actual | Calculated | Measurement | Diff. x Path |
|---|---|---|---|---|---|---|
| 1 | 99_ETOH | Calibration | 99.00 | 98.15 | 2.9263 | -0.85 |
| 2 | 85_ETOH | Calibration | 85.00 | 87.56 | 2.4914 | 2.56 |
| 3 | 75_ETOH | Calibration | 75.00 | 72.89 | 1.8888 | -2.11 |
| 4 | 62.5_ETOH | Calibration | 62.50 | 61.85 | 1.4352 | -0.65 |
| 5 | 50_ETOH | Calibration | 50.00 | 51.03 | 0.9907 | 1.03 |
| 6 | 25_ETOH | Calibration | 25.00 | 25.02 | -0.0775 | 0.02 |
| 7 | Commercial sample 1 | Validation | 62.00 | 61.99 | 1.4411 | -0.01 |
| 8 | Commercial sample 3 | Validation | 70.00 | 69.80 | 1.7619 | -0.20 |
| 9 | commercial sample 4 | Validation | 70.00 | 70.04 | 1.7717 | 0.04 |
| 10 | Commercial sample 2 | Validation | 62.00 | 61.99 | 1.4411 | -0.01 |

**Figure 4. Calibration result of ethanol% measurements**

has a high-performance optical engine, an integrated computer and a LED light bar for visual indication.

Additionally, the Nicolet Summit PRO FTIR spectrometer can be combined with the Thermo Scientific™ Everest™ Diamond ATR accessory for routine QA/QC in alcohol-based hand sanitizers production. This accessory consists of a monolithic diamond ATR crystal combined with four single-bounce crystal plates. The Everest accessory uses a smart chip in combination with OMNICParadigm software to automatically recognize the accessory and optimize the measurements. Since ATR provides a precise and non-destructive sampling method, without the need for sample preparation, it is perfect for fast and accurate analysis of incoming materials or finished products.

## Confirmation of alcohol-specific materials

This section will describe the three applications that were developed on the Nicolet iS50 FTIR spectrometer and rapidly deployed to the Nicolet Summit FTIR spectrometer. An OMNIC Paradigm software workflow can be used to identify any incoming alcohol by combing search, QCheck and TQ Analyst applications (Figure 7).

**Figure 5: Nicolet Summit PRO FTIR Spectrometer with Everest ATR accessory and on-board touchscreen monitor**

A workflow available in the OMNIC Paradigm software allows the operator to decide the category of the incoming sample and choose between two workflows for analysis. *Incoming Material Identification* can be used to check that the incoming material is as specified on the packaging, whereas *Final Product Confirmation* verifies that the product has the same spectral fingerprint as an approved reference and/or ensures that significant levels of methanol are not present. Additionally, multiple samples can be easily analyzed using the loop function present in the workflow.

The QA/QC analyst needs to verify that the incoming material is labeled correctly – QC Compare can do this by comparing the sample spectrum with a reference spectrum, such as ethanol. The Nicolet Summit can measure samples and compare them to different spectra from the *Material Identification Library* (i.e., ethanol, methanol, 1-propanol and 2-propanol) using the OMNIC Paradigm Software workflow.

The *Final Product Confirmation* category by QCheck spectral correlation, is the optimal way to verify the quality of finished products, as they can be analyzed for contamination. Figure 8 is a report from an experiment of a commercial hand sanitizer contaminated with methanol. Since methanol reduces the spectral correlation below the allowable threshold of 99, this adulterated sample failed the QCheck finished product verification.

In addition to sample inspection, FTIR can be used to quantitatively determine the alcohol percentage in final products. In one experiment, a series of ethanol/water solutions with ethanol varying from 25–99% (v/v) were measured by FTIR and used to construct a calibration curve using Lambert-Beer's Law in TQ Analyst Software. The calibration curve exhibits excellent linearity with a correlation

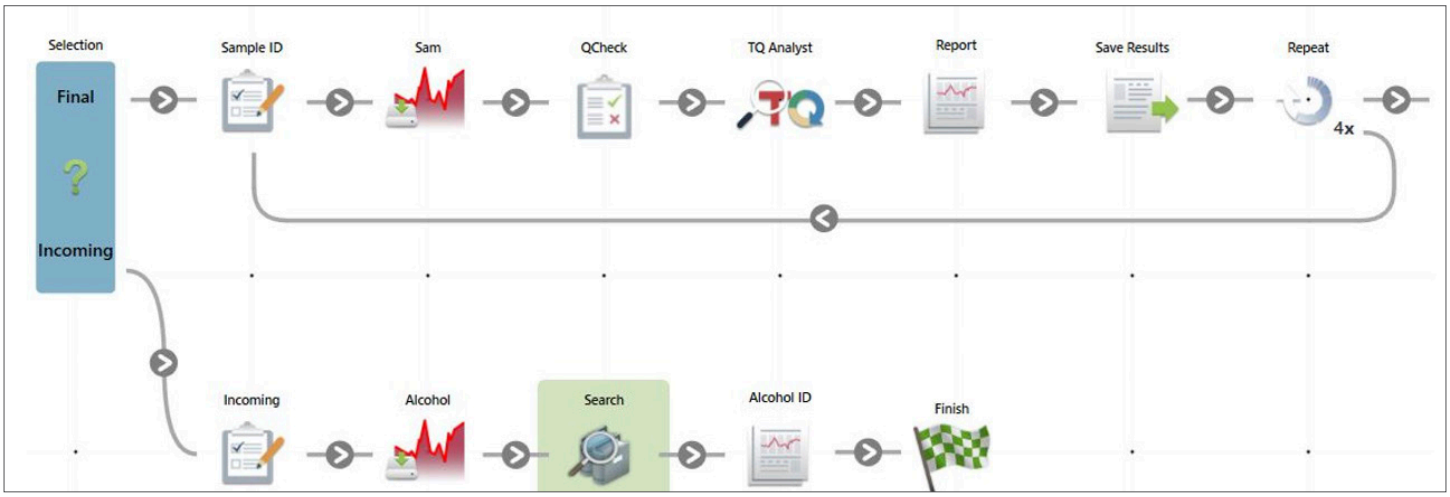



**Figure 6: Workflow to verify the final product and to confirm incoming alcohol identification**

coefficient higher than 0.998. TQ Analyst software was used for determining the accuracy of the measurements of five repeat ethanol-based hand sanitizer samples on the Nicolet Summit instrument (Figure 9). A concentration of 69.58% ethanol was determined, with a standard deviation of 0.20.

Another important step during QA/QC production is the identification and quantification of isopropanol and possible contaminants such as methanol, as it can cause toxicity

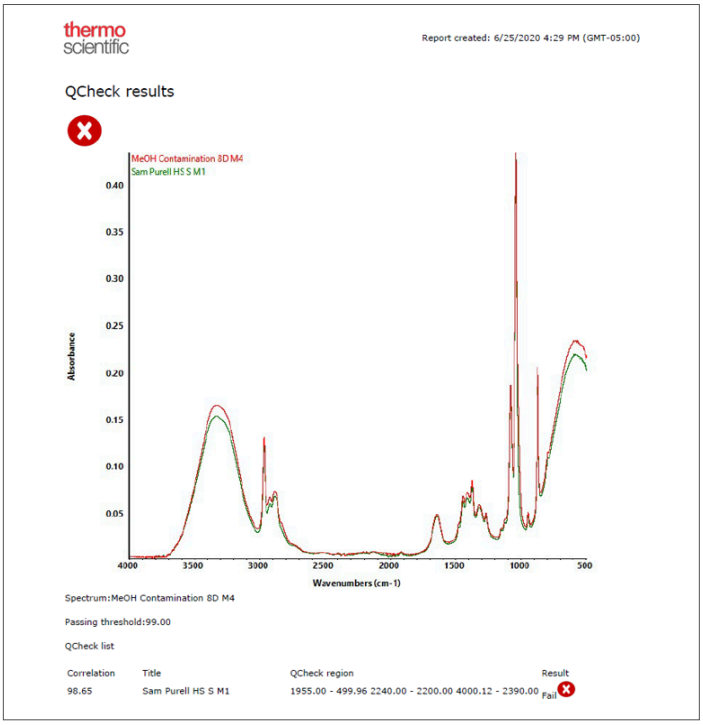



**Figure 7: Results of applying QCheck to a methanol adulterated (red trace) hand sanitizer sample**

when absorbed through the skin or ingested.[10] Additionally, the isopropanol concentration must be balanced correctly; although high concentrations are not needed to denature proteins, concentrations lower than 60% will not kill germs.[2] The quantification of methanol and isopropanol weight percentage in the sample was recently included in the TQ Analyst software. As an example, Figure 9 shows the analysis of a denatured hand sanitizer sample. The term *denaturated* refers to an ethanol solution containing specific additives to alter its odor and/or taste to dissuade consumption. The use of additives is strongly discouraged, while methanol is prohibited.[5]

ATR FTIR measurements take less than 30 seconds without the need for sample preparation. Rapid quality control of materials at different production steps can be done with QCheck and QC Compare, increasing consumer and production confidence in the final product quality. The precise and quantitative determination of the alcohol content in final formulations can be performed by TQ Analyst software. Therefore, a single run of the OMNIC Paradigm workflow on the Nicolet Summit FTIR spectrometer (equipped with an Everest Diamond ATR accessory) is ideal for QA/QC of alcohol-based hand sanitizers.

## Creating workflows in OMNIC paradigm software

Workflows can help optimize and automate repeatable analysis tasks, minimizing errors and increasing overall efficiency. Using the OMNIC Paradigm software an application package containing all the workflows required for any specific task can be created.

| Repeat Precision | | | |
|---|---|---|---|
| Sample ID | % ETHANOL | Qcheck | Date and time |
| | | | |
| HandSanitizer S5 M1 | 69.24 | 98.89 | 18 Jun 2020 09:37:52 (GMT-05:00) |
| HandSanitizer S5 M2 | 69.59 | 98.86 | 18 Jun 2020 09:37:52 (GMT-05:00) |
| HandSanitizer S5 M3 | 69.70 | 98.93 | 18 Jun 2020 09:37:52 (GMT-05:00) |
| HandSanitizer S5 M4 | 69.74 | 98.9 | 18 Jun 2020 09:37:52 (GMT-05:00) |
| HandSanitizer S5 M5 | 69.65 | 98.81 | 18 Jun 2020 09:37:52 (GMT-05:00) |
| | | | |
| **Average** | **69.58** | | |
| **Standard Deviation** | **0.20** | | |

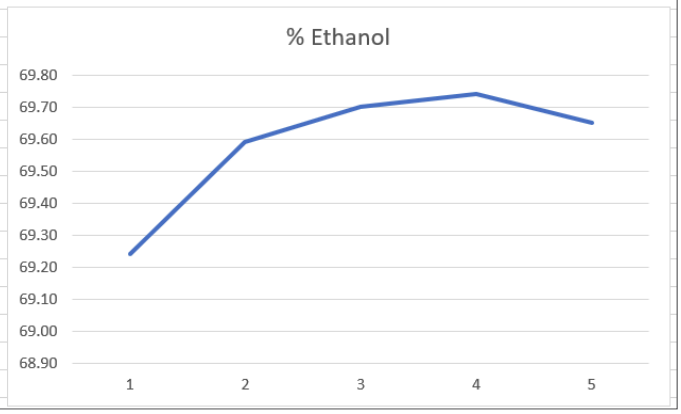



**Figure 8: Chart of TQ Analyst Simple Beer's Law method results appended to readable CSV file by OMNIC Paradigm workflow. Software like Excel or Origin may be used for statistical analysis and to build a custom control graph**

Before any sample analysis, it is recommended that an operator does a performance check, crystal clean, and background:

1. *Performance Check,* measures necessary performance parameters of the FTIR spectrometer like instrument noise and spectral quality.
2. *Crystal Clean,* cleans the ATR crystal before analysis to minimize cross-contamination.
3. *Background,* measures the signal of the instrument with no sample present. This is important to check that the equipment is working well and to subtract ambient noise and signals that contribute to the measurement.

The next set of workflows can be used to measure the hand sanitizer production process:

1. *Incoming QC,* checks the incoming material matches the alcohol specified on the packaging
2. *Product Check,* verifies that the production lot has the same spectral fingerprint as the approved reference sample.
3. *Percent Ethanol,* determines the percent ethanol in the final product to verify that it meets regulatory requirements.

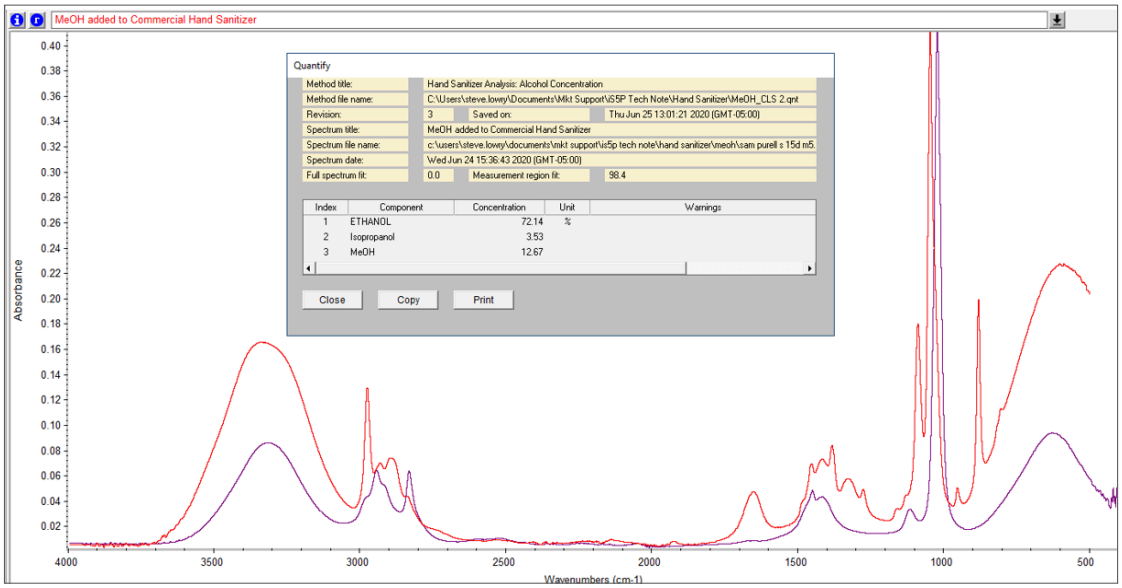



**Figure 9: TQ Analyst method calculates weight percentage of methanol and isopropanol in hand sanitizer sample**

The OMNIC Paradigm software provides an easy way to use, create, and edit workflows. User-defined decisions, loops, images, and instructions can be combined to created customizable workflow packages to be used in specific analysis types. Moreover, the software enables end-users to access specific workflows inside a package, resulting in great process control and repeatable results. All these characteristics make OMNIC Paradigm software a potent tool for QA/QC laboratories.

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