# The Agilent Cary 630 FTIR Spectrometer Quickly Identifies and Qualifies Pharmaceuticals

## Application Note

Small Molecule Pharmaceuticals


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## Introduction

In the pharmaceutical industry, the correct identification and proper qualification of raw ingredients (actives and excipients), in-process materials, and final products are important tasks in quality control and quality assurance. In addition, due to increasing globalization of raw material supplies, there is significant interest in rapid means for ensuring purity and authenticity at specific checkpoints, to prevent substandard, counterfeit, contaminated, or incorrectly labeled ingredients from entering production.

FTIR spectroscopy is useful since it provides a fast and nondestructive means for accurate identification and qualification of materials, to ensure acceptability. For example, simple spectrum-matching methods such as infrared library searching can be used for positive identification of a given unknown sample. Library search uses a mathematical algorithm to measure a correlation between a given material spectrum and the available reference spectra in the library database. The library search method can detect differences in the 5 to 10 % range between samples and analogous reference spectra. However, for qualification, a more thorough and rigorous analysis is required to distinguish between similar types of materials, particularly those with differences of 1 to 5 %.

This application note describes a very sensitive classification method, partial least squares–discriminant analysis (PLS-DA), incorporated in Agilent innovative MicroLab software, to classify and qualify materials of interest with greater sensitivity and specificity than possible with simple library search methods. We demonstrate the qualification of pure active material (acetylsalicylic acid) from acetylsalicylic acid contaminated with three different excipients. This sensitive technique is suitable for qualifying acceptable material, as well as identifying out-of-specification materials.

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## Instrumentation

This project used an Agilent Cary 630 FTIR (Figure 1), a multipurpose high-performance compact FTIR spectrometer suited to QA/QC or method development applications in the pharmaceutical industry. The spectrometer meets the specification requirements published by US, European, Japanese, Chinese, Indian, and International Pharmacopeia [1]. The spectrometer provides major advantages for routine measurements in quality control/assurance due to its ease-of-use, ruggedness, compact size (portability), and pre-aligned sampling interfaces designed for the system.

*Figure 1. Agilent Cary 630 FTIR instrument with ATR sampling accessory attached.*

We used the single reflection diamond attenuated total reflectance (ATR) sample interface, which is appropriate for the analysis of pharmaceutical materials as it requires no sample preparation. A high-quality spectrum is obtained simply by placing the sample on the diamond sensor, and using the sample press to ensure good contact. The diamond ATR sensor is impervious to abrasion, requires very small amounts of sample, and is easy to clean between samples. The use of ATR for the analysis of pharmaceutical materials has been cited in USP [2].

## Advantage of Agilent Cary 630 and Agilent MicroLab Software in Pharmaceutical Applications

- The Cary 630 FTIR wavelength accuracy, resolution, and other specifications meet or exceed requirements published by US, European, Japanese, Chinese, Indian, and International Pharmacopoeia.
- The Cary 630 FTIR is available with ATR, diffuse reflectance, and transmission-sampling interfaces specified in the US and other pharmacopoeia for identification purposes.
- The Cary 630 FTIR requires minimal maintenance, and occupies little space on lab benches or loading docks.
- Agilent 21 CFR Part 11 MicroLab PC software in the Cary 630 FTIR provides the data security and logging capabilities required by the pharmaceutical industry.
- Automated installation qualification/operational qualification (Auto IQ/OQ) software can be used to routinely verify instrument performance.
- MicroLab software is method driven, highly visual, and intuitive, which minimizes training costs, reduces the risk of user-error, and allows less experienced users to get up and running immediately.

Two different batches of pure acetylsalicylic (≥ 99 %) acid were obtained from the same vendor. The acetylsalicylic acid from the first batch was contaminated with three different common excipients; corn starch, microcrystalline cellulose, and lactose monohydrate. Each excipient, at weight percentages ranging from approximately 1 to 20 %, was added to the pure acetylsalicylic acid separately to create calibration and validation standards. A total of 14 spectra, obtained from different samples from the two batches of pure acetylsalicylic acid, was used to represent the pure class. Similarly, a sample set of 18, 13, and 19 spectra of acetylsalicylic acid contaminated with corn starch, microcrystalline cellulose, and lactose monohydrate was used to represent the impure class of acetylsalicylic acid.

The spectra of pure and contaminated acetylsalicylic acid were recorded as-is without any grinding, using a Cary 630 FTIR spectrometer equipped with a single bounce diamond ATR sampling accessory. Each spectrum, recorded in the spectral range 4,000 to 650 $cm^{-1}$, is the co-addition of 74 scans at 4 $cm^{-1}$ resolution with a total measurement time of 30 seconds.

The validation standards consisted of pure acetylsalicylic acid and material contaminated with excipients that were included in the calibration model, as well as materials (magnesium stearate, methylcellulose, calcium carbonate, and salicylic acid) that were not included in the calibration sample. The classification method was based on PLS-DA using mean centering and a nine-point Savitzky-Golay first derivative as the preprocessing algorithm.

## Results and Discussion

PLS-DA is a supervised classification technique, and is considered more sensitive compared to principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) when separating spectra that are nearly identical [3]. To build a PLS-DA calibration model, representative spectra of samples for each class of compound are required. To have a robust calibration model for a specific raw ingredient, calibration set samples representing pure samples should encompass sources of variation normally encountered for that particular material, such as samples from multiple vendors, batches, or manufacturing processes.

In most PLS-DA classifications, one target group is separated against the others. However, we used a group of spectra of pure acetylsalicylic acid discriminated separately against each type of contaminated acetylsalicylic acid. The main advantage of building separate calibrations was the ability to differentiate lower percentage contaminated samples from the pure acetylsalicylic acid.

Figure 2 shows the classification results using cross validation for a group of spectra of pure acetylsalicylic acid versus each contaminated sample. Five, six, and five factors were required for the proper separation of pure acetylsalicylic acid from the acetylsalicylic acid contaminated with cornstarch, microcrystalline cellulose, and lactose monohydrate, respectively.

Figure 2 shows that the data set was clearly divided into two different groups. Arbitrary values of 10 and 20 were given for a pure sample and the contaminated samples, respectively, when applying PLS regression for the discrimination. A threshold (y-value) based on distribution assumption from the plot was chosen to set the condition on MicroLab software for the classification and qualification of pure versus impure acetylsalicylic acid.

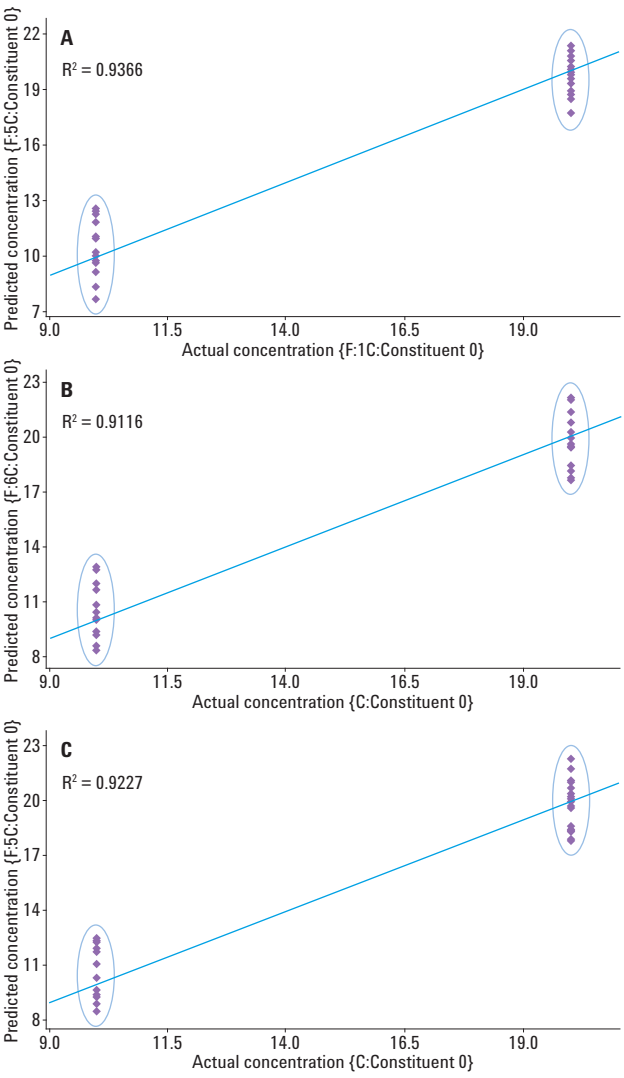


*Figure 2. PLS-DA separation of pure acetylsalicylic acid against acetylsalicylic acid contaminated with corn starch (A), microcrystalline cellulose (B), and lactose monohydrate (C).*

The separate models were combined into a single method using the unique logic-setting feature of MicroLab to classify and qualify the unknown validation samples as pure or impure acetylsalicylic acid (Figure 3).

The final method provided 100 % correct classification of the tested samples, predicting whether the samples were pure, or contained contaminants as low as 0.5 %. The implemented method permits highly informative and simple visual display of results. Samples with acceptable results are highlighted in green, whereas impure samples are highlighted in red, as shown in the result screen of the MicroLab method (Figure 4).

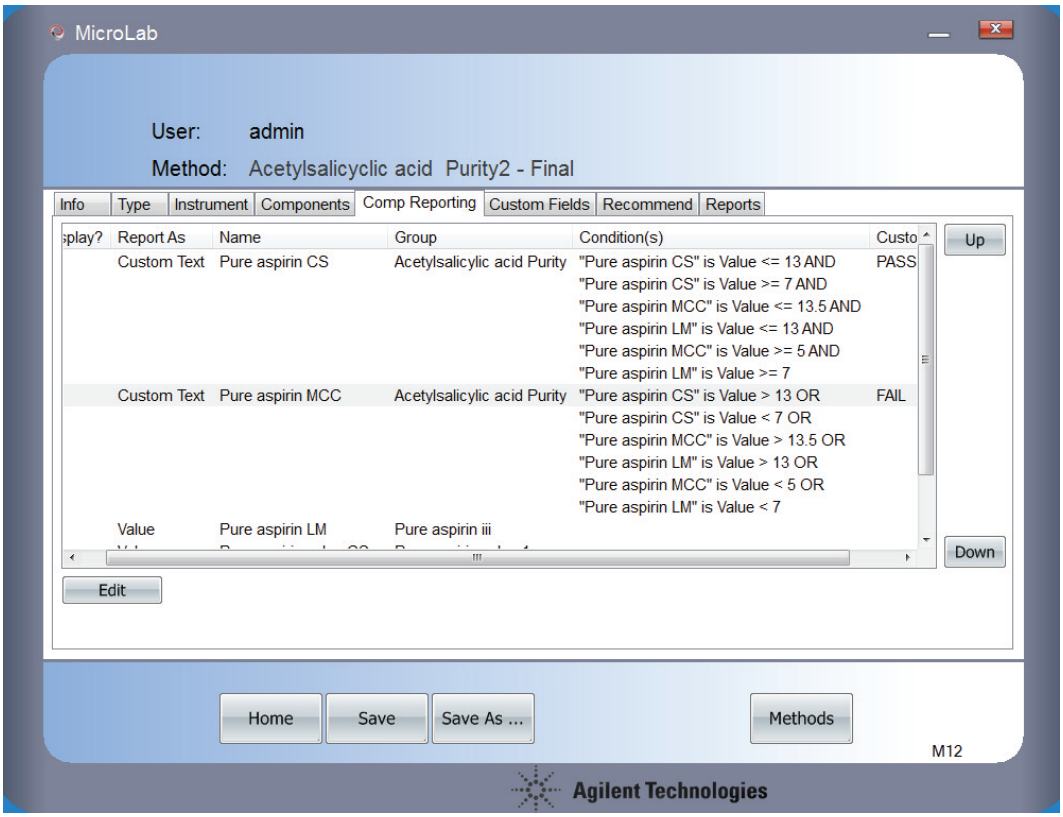


*Figure 3. Agilent MicroLab software logic-setting feature for reporting a component using conditions required for the proper discrimination and result display.*

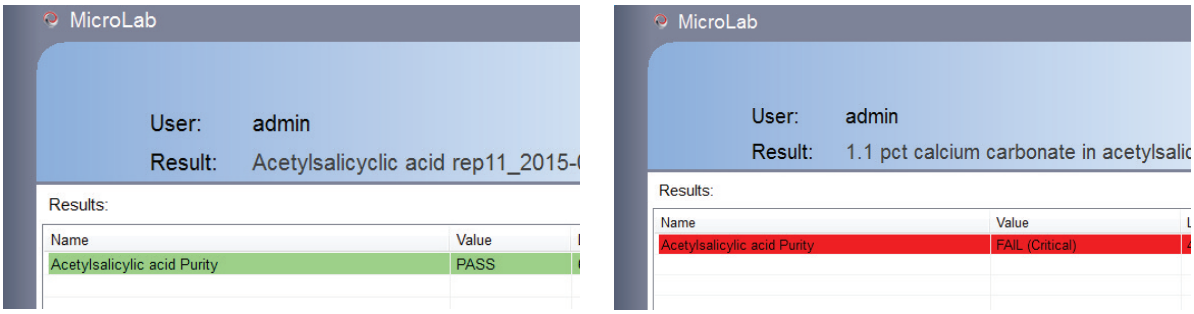


*Figure 4. The final method in Agilent MicroLab software showing the result of the purity of acetylsalicylic acid as either pass or fail, which can be used in qualifying the material for further application.*

## Conclusions

We showed that the Agilent Cary 630 FTIR spectrometer with ATR sampling technology and a PLS-DA method was suitable for analyzing, characterizing, and verifying raw materials and ingredients in pharmaceutical applications.

The method developed here uses the combination of the sensitive PLS-DA classification technique combined with the exclusive logic-setting capability of Agilent MicroLab software to achieve high sensitivity and specificity for the qualification of pure actives, excipients, and mixtures. Similar methods for qualification can easily be developed for other ingredients of interest used in pharmaceutical product development.

Keeping out-of-spec raw materials from entering the manufacturing process is critical to support product quality and safety. The Cary 630 FTIR effectively meets the need to verify the identity and specifications of pharmaceutical materials, whether spectral library search, discriminant analysis, or quantitative analysis are required for the specific application [4].

## References


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