

Food Authenticity Testing with the Agilent 6546 LC/Q-TOF and MassHunter Classifier

Authors

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Abstract

False labeling and adulteration are growing problems in food manufacturing, underlining the need for easy-to-use tools for quality control analysis in this industry. This Application Note describes a novel method enabling routine testing of food authenticity. The workflow consisted of an Agilent 6546 LC/Q-TOF used with MassHunter Profinder 10.0, Mass Profiler Professional 15.0, and Classifier 1.0 software. The method rapidly produced reliable results as insights into food quality.

Introduction

The food manufacturing industry is increasingly interested in food authenticity testing, as adulteration and fraudulent labeling becomes more common across a complex food supply chain. With the costs of premium ingredients and products rising, the incidence of adulterated or imitation products will continue to increase. Currently, the development and deployment of methods and procedures for testing for such activity is limited by the lack of user-friendly tools. Advancing such tools and workflows can improve quality control procedures of ingredients in the supply chain or final manufactured products in the consumer market place. This advance allows food manufacturers to consistently use authentic materials.

Mass spectrometry (MS) allows for the measurement and profiling of molecular components of food stuffs. These profiles can be used to classify a sample and determine if it is authentic or adulterated with high precision and accuracy. The Agilent 6546 LC/Q-TOF mass spectrometer has significantly improved low mass resolution with simultaneous broad dynamic range. These features allow more features to be found and measured in a complex sample. Analysis software and workflows for development and use of food authenticity tests have required highly trained specialists, making it challenging for food labs to get involved.

This Application Note introduces software for the development and implementation of a complete authentication workflow, from sample preparation to data analysis (Figure 1). Fast sample preparation is performed with a QuEChERS kit, while analyte separation and detection are accomplished with an Agilent 1290 Infinity II LC and 6546 LC/Q-TOF. An authenticity model can be built from a

scientist's data processing, which can be automated with MassHunter Profinder 10.0 and Mass Profiler Professional (MPP) 15.0. Agilent MassHunter Classifier 1.0 performs automated authenticity analysis of samples to streamline results. With these improved tools, routine food authentication analyses can widely and easily be implemented.

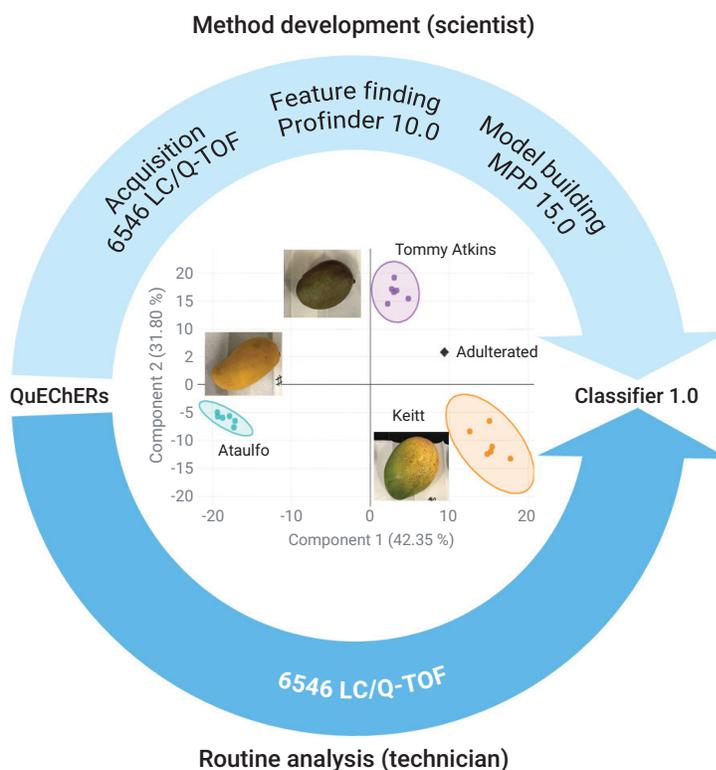


Figure 1. A complete authentication workflow for the method developer and the routine analyst. The process begins with QuEChERS sample extraction and cleanup. In the method development procedure (top), data for the samples are acquired using a 6546 LC/Q-TOF. These data are analyzed using much improved Profinder 10.0 for feature extraction, and MPP 15.0 for building the model. The developer can test quality control samples and unknowns using their classification model in Classifier 1.0. In routine use, the Classifier software is simple to use, allowing an analyst to run and review samples productively (bottom). Result review includes a plot (center) to easily see if a sample is pure (colored circles) or adulterated (shown here, black diamond).

Experimental

Sample set, preparation, and data acquisition

Certified authentic samples are needed for building a model. For this study, three different mango varieties were analyzed: Ataulfo, Keitt, and Tommy Atkins. Although these were not certified authentic, they have unique phenotypes that were used to correctly identify them, which is acceptable for this proof-of-concept study. Six biological replicates, or individual mangos per variety, were collected from local markets in the California region. The mangos were peeled, and the meat was homogenized. The homogenate was then processed with a QuEChERS EN protocol¹. In a 50-mL conical tube, 10 g of mango homogenate was mixed for two minutes with 10 mL of acetonitrile. An EN salt pouch was added and shaken for two minutes. Then, the sample was centrifuged for six minutes at 3,500 rpm. The upper layer was recovered and stored at 7 °C in a glass HPLC vial until analysis.

A positive quality control (QC), or pure sample, for each variety was made by mixing the homogenate from the six biological replicates (Figure 2).

Adulterated samples, or negative controls, were prepared by mixing the positive QC samples at known ratios, for example, 20:80 and 50:50. The QC samples were prepared with QuEChERS EN, and stored in the same way as the individual samples.

Samples were analyzed with a 1290 Infinity II LC coupled to a 6546 LC/Q-TOF. Using an injector program, 2 μ L of sample was aspirated followed by a needle wash. Next, 1 μ L of internal standard (IS), 100 ppb deuterated pesticide mix, was aspirated,

the needle was washed, then all the sample and IS was injected. This internal standard allowed for the data quality to be monitored over the entire experiment. The Q-TOF was tuned and calibrated in positive mode (m/z 1,700 range).

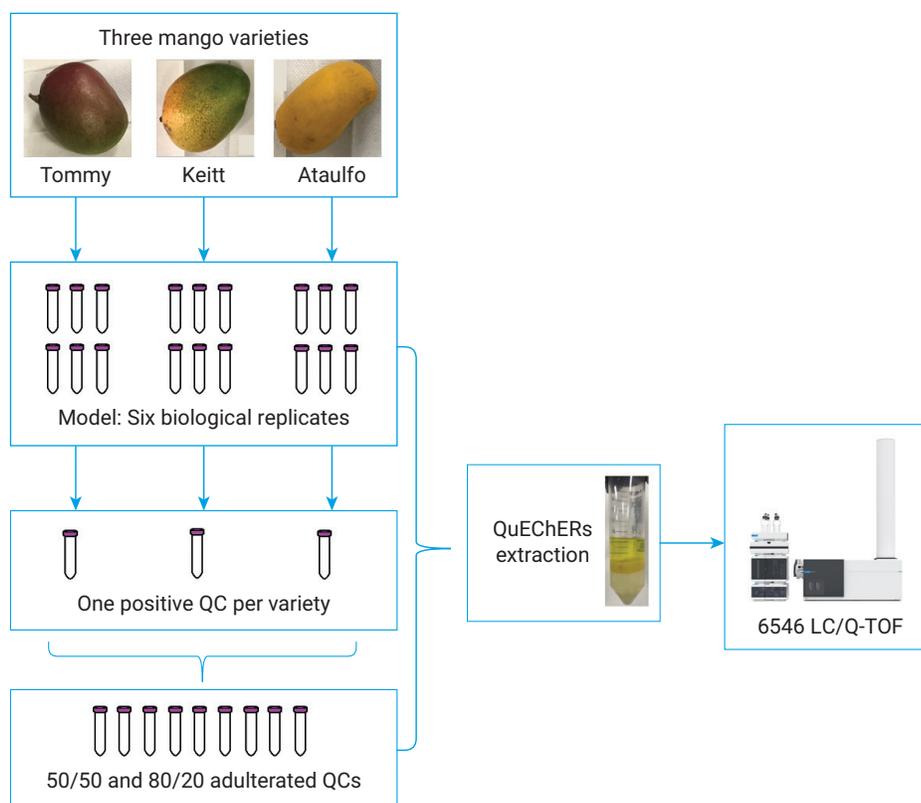


Figure 2. Sample and laboratory workflow for building a mango authenticity model. Six replicates of three mango classes were used to make the positive QC samples. Adulterated mixtures were made by mixing the pure QCs. Each sample was processed with the QuEChERS kit, and data were acquired using a 6546 LC/Q-TOF.

Over five days of continuous acquisition, excellent mass accuracy was achieved with two internal reference masses, purine and HP-921 (^1H , ^1H , ^3H -tetrafluoropropoxy phosphazine). MS data acquisition rates were set to maintain a minimum of 8 to 12 data points across chromatographic peaks. Table 1 shows additional method details.

The model samples (six biological replicates \times three mango varieties) were randomized in the worklist, followed by the injection of randomized adulterated samples. This was intended to mimic a typical laboratory workflow where model data are collected followed by the unknown samples. In the worklist, after every 10 sample injections, the three positive QC samples were injected in a random order. To assess method and model longevity, 14 days after the data collection, freshly prepared positive QCs and adulterated samples were analyzed using the same method and model.

Results and discussion

Data quality

The mass accuracy and area of the deuterated internal standards are plotted in Figure 3. These results serve as a quality control check for not only every injection, but also the entire dataset. These data showed that throughout the entire experiment, mass error on the 6546 LC/Q-TOF was low (<2 ppm), and had a stable signal (<10 % RSD). The retention time drifted only 0.1 minutes throughout the whole worklist. This data reproducibility gives confidence in the instrument performance of each run.

Table 1. Acquisition details for the 6546 LC/Q-TOF analysis.

Acquisition Parameters	
Column	Agilent ZORBAX SB-Aq, 3.0 \times 150 mm, 3.5 μm
Mobile Phase A	Water + 0.1 % formic acid, 5 mM ammonium formate, 0.5 mM ammonium fluoride
Mobile Phase B	Acetonitrile + 0.1 % formic acid, 5 mM ammonium formate, 0.5 mM ammonium fluoride
Sheath Gas Temperature	400 $^{\circ}\text{C}$
Sheath Gas Flow	12 psi
Gas Temperature	325 $^{\circ}\text{C}$
Gas Flow	10 psi
Nebulizer	20 psi
Capillary Voltage	4,000 V
MS Tune	m/z 1,700
MS Mode	Positive
Acquisition	MS only
MS Range	m/z 50 to 1,000

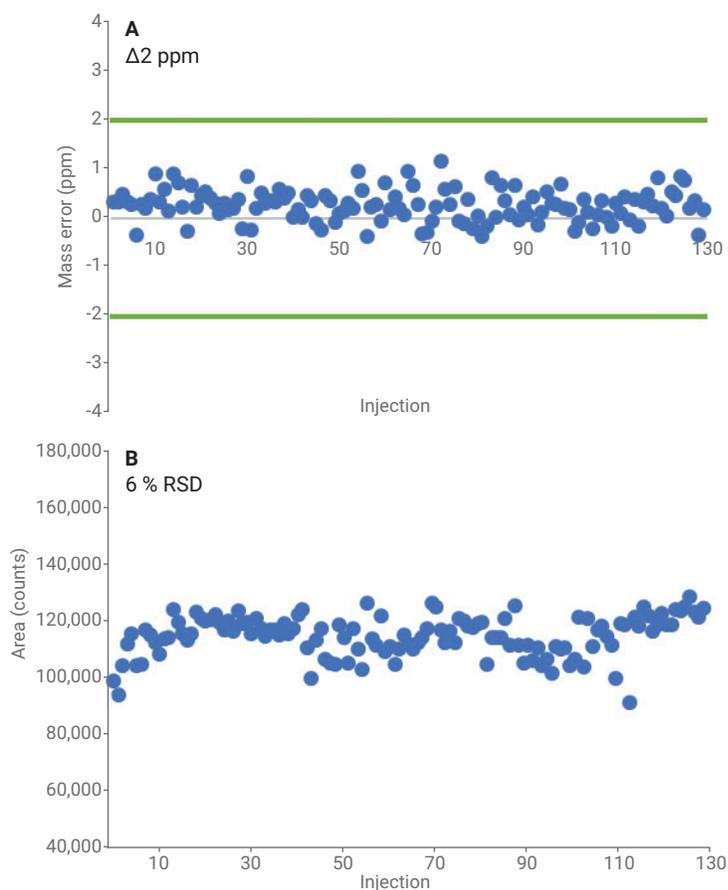


Figure 3. Dimethoate- d_6 (m/z 236.0446, RT 10.4 minutes) results during the entire analysis. The mass error for 100+ injections had less than 2 ppm error, and the area was stable with 6 % relative standard deviation (RSD). This is a representative result of six internal standards.

Method development workflow: Profinder 10.0 and MPP 15.0

For food authenticity, the goal of the analysis is to find robust identifiers, not every differentiator. This strategy should allow for an analysis model to be used for an extended period without the need of a model update. There are many key differences between this Profinder and MPP workflow compared to other types of analysis (for example, metabolomics).

The 18 model samples were loaded into Profinder 10.0, and grouped by their mango variety. Because of the minimal retention time drift in this dataset, no retention time correction was needed for this analysis. The batch recursive feature extraction (small molecules/peptides) wizard was selected to detect features in an untargeted manner. A few changes were made to the default method. The protonated ion species was selected with the *common organic molecules (no halogens)* isotope model and a charge state limit of 1. A height filter of 3,000 was used whenever requested by the wizard. Finally, the molecular feature extractor (MFE) algorithm and the target score for feature quality was increased to 80.

The method was saved with a unique name to be used later in the Classifier 1.0 software.

The untargeted analysis found over 4,000 features (entities), and these results were exported as a Profinder Archive (.PFA) file for import into MPP. Once imported into MPP, the data were filtered by frequency, retention time, ANOVA statistical analysis statistics, and fold change (Figure 4). Once the analysis was complete, a partial least square discriminant analysis (PLS-DA) model was created. The PLS-DA plot and groupings were inspected for goodness of fit (R^2), predictive power (Q^2), then exported as an MPP model.

Routine analysis workflow: Classifier 1.0

The development process ensures that the routine lab has an effective way of extracting features from samples (Profinder), and processing them to get a model (MPP). For routine classification of newly acquired samples, Profinder and MPP are not amenable for quick and easy analysis because they require a high level of expertise. Classifier 1.0 is innovative software that allows an analyst to take saved Profinder and MPP methods and apply them to new samples and generate a fast result. This software

does not require the analyst to use Profinder and MPP.

Classifier has a simple interface to input the method and sample information (Figure 5). Once samples are submitted, additional samples can be added to a project at any time during the Classifier analysis. Each sample takes a few minutes to analyze, and data can be reviewed as the processing moves down the sample queue. A project, containing one or more sample results, can be saved, re-opened, or exported as a report.

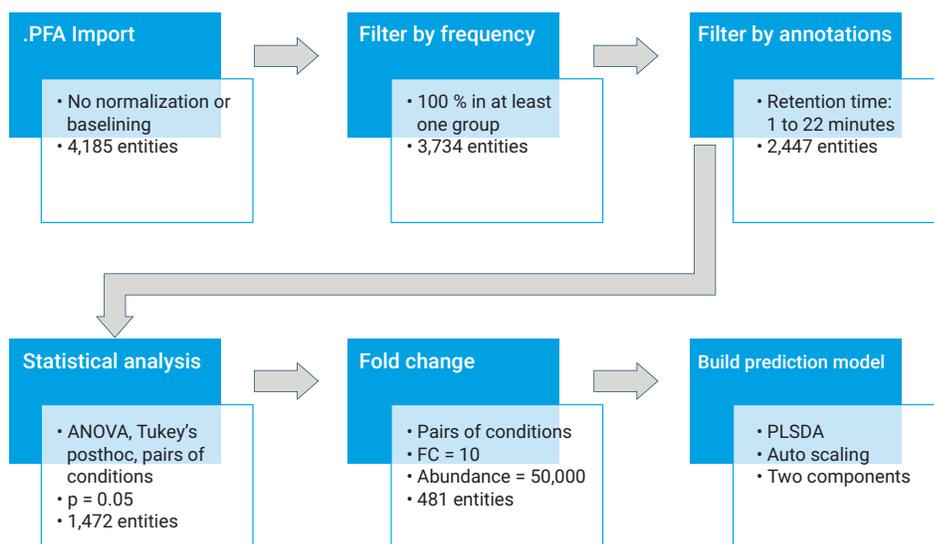


Figure 4. MPP analysis workflow and key parameters. At each filtering step, the number of entities remaining is noted.

The screenshot shows a 'New Project' dialog box with the following fields and options:

- Project Name: Project
- Samples Type: (.d) (.cef)
- Profinder Method: [Select]
- MPP Model: [Select]
- Samples: [Select]
- Buttons: Submit, Cancel

Figure 5. The Classifier 1.0 new project user interface is simple and easy to use. The Profinder method and MPP model, which are created during method development stage, are selected. One or more samples can be added.

For this study, the MPP model, Profinder method, and all the adulterated samples were added to a Classifier 1.0 project. Results were populated in the Sample Table (Figure 6A) where each row contains the sample name, predicted class, and confidence value associated with the classification. Pending samples that are not yet analyzed remain queued in the Sample Table until results are available (Figure 6B). To review data, a sample can be selected from the Sample Table. This selection updates the interface to show the reviewed sample's location in the model's principal

component analysis (PCA) plot as a black diamond. This plot contains the model samples within Hotelling ellipses, or 95 % confidence ellipses (Figure 6C). For every sample being analyzed, the individual features that belong to the model can also be reviewed in the Compound Table (Figure 6D).

In this analysis, the mango QC data were not included in the model building, but rather were analyzed in Classifier 1.0 as a QC check for authentic samples. Each QC sample was classified correctly, and the PCA plots matched the correct variety for the QC. The six biological

replicates that created the model are tightly contained in Hotelling ellipses (Figure 7). The review sample is shown as a black diamond in the PCA plot. The sample's position relative to the Hotelling's are indicative of their purity, as is the confidence value listed in the Sample Table (Figure 6A). When the sample is a pure QC, the black diamond is in or very near the mango grouping to which it belongs (Figures 7A and 7B). When the sample is adulterated, the black diamond is plotted further away from the grouping (Figures 7C and 7D).



Figure 6. Classifier 1.0 results are displayed in the Sample Table (A) and unanalyzed samples are queued (B). The individual sample details can be viewed by PCA plots (C) or feature details in the Compound Table (D).

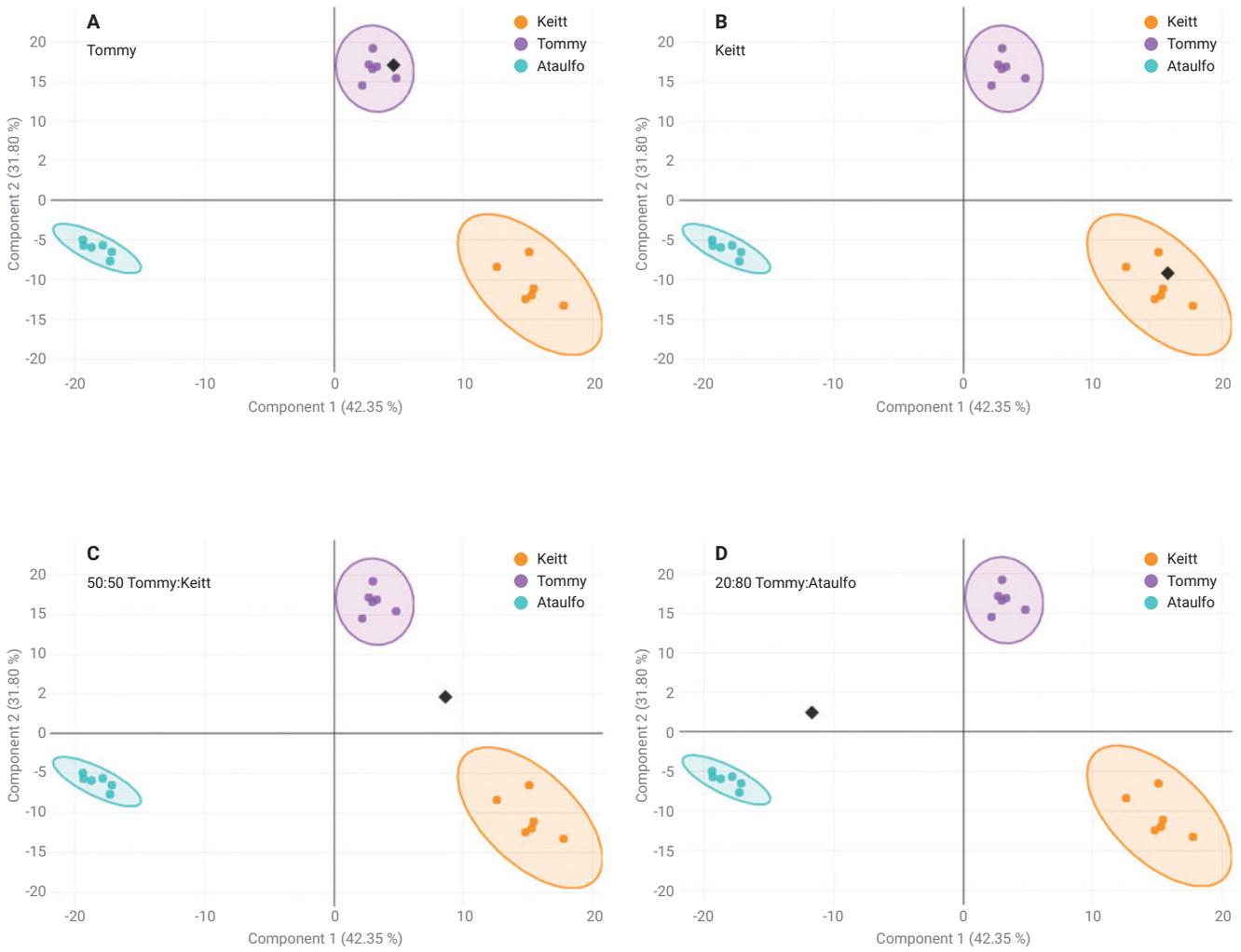


Figure 7. Classifier 1.0 PCA plot results. The Keitt class is orange, the Tommy Atkins class is purple, and the Ataulfo class is teal. The black diamond represents the selected sample. The pure samples, (A) Tommy and (B) Keitt, have the sample fall directly within their group. The adulterated sample, (C) 50 % Tommy: 50 % Keitt and (D) 20 % Tommy: 80 % Ataulfo, are not near the model groupings.

Figure 8 summarizes the confidence results for this study. Pure QC samples have high confidence values, whereas adulterated samples and negative controls have lower confidence values. In this case, a cutoff of 0.8 would have 100 % accuracy for correctly identifying a sample as pure or adulterated based on the confidence value alone. Furthermore, the method holds good results for an extended series of analyses because the same results were achieved for samples collected on day 1 and day 14. The precision of the method is also very high, with an RSD <5 % for the confidence value (n = 10).

New workflow improvements for the method development scientist

A method automation tool in MPP 15.0 allows a method development scientist to efficiently create methods, and pass them off for routine analysis. The tool, shown in Figure 9, has easy drag-and-drop selection for building the method from a list of analysis options. Once the method is built, it can be saved and used on any new .PFA, eliminating

the need to click through all the steps in the Workflow menu. This software improvement also makes it easier to add new authentic samples to a model since the saved analysis method can easily be re-applied. A statistical analysis method, such as the one in Figure 4, can be reprocessed with additional authentic samples to generate an updated model in just a mouse click with less time and human error.

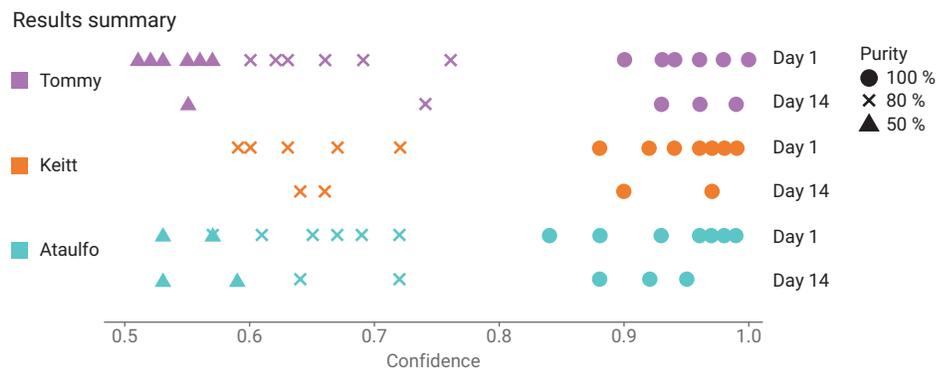


Figure 8. Summary plot of mango results from Classifier 1.0. The color represents the mango variety classification for each sample. Data from day 1 and day 14 experiments are plotted.

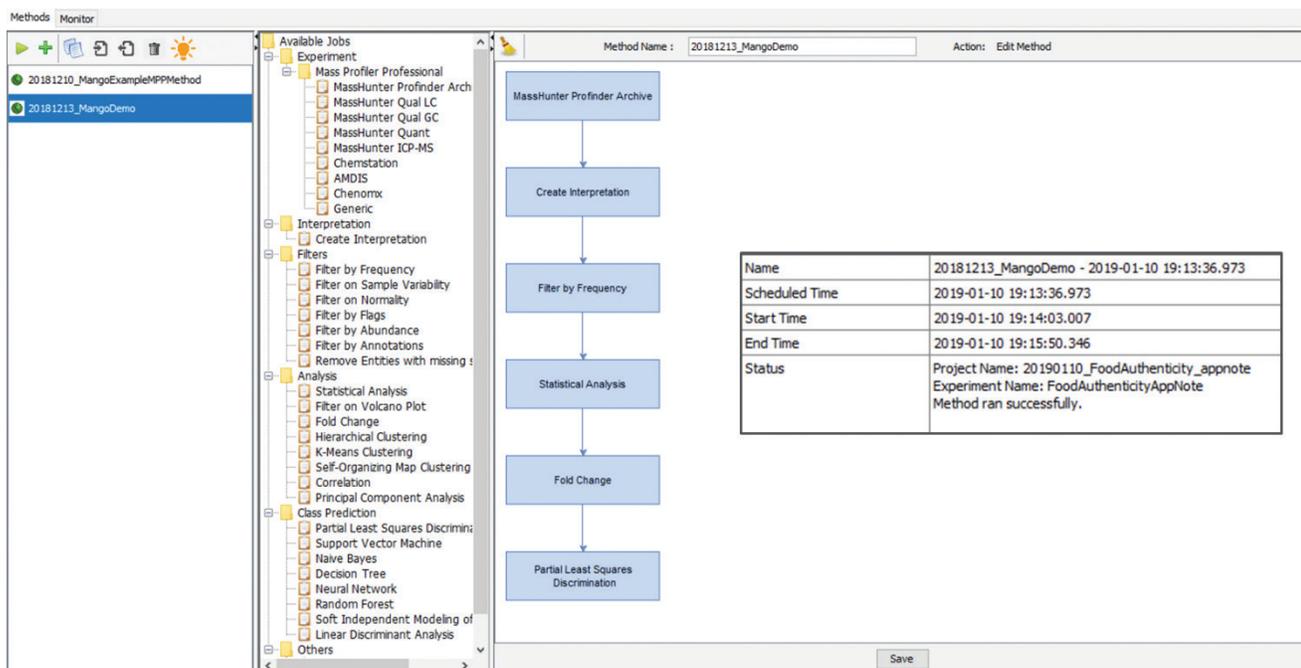


Figure 9. MPP 15.0 method automation user interface with new features. The left panel is a list of methods with a play button to initiate the desired method. New methods can be built on the right side by selecting steps for the middle panel to be added to the method. Using method automation makes the analysis faster and provides a report when complete (inset).

Conclusion

Food authenticity workflows are needed to make food testing more routine as adulterated and mislabeled products and ingredients become more common. This desired workflow is realized with the 6546 LC/Q-TOF, Profinder 10.0, MPP 15.0, and Classifier 1.0. A method development scientist has a faster, automated workflow to help build a model. For routine analysis, an analyst only needs to use Classifier 1.0 for fast classification from new data. The workflow produces fast, clear, and reliable results for labs to explore the quality of their ingredients and products.

Reference

1. <https://www.agilent.com/en/products/sample-preparation/sample-preparation-methods/quechers/extraction-kits-preparation-methods/quechers/extraction-kits>

www.agilent.com/chem

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