White paper

Raman handheld analyzers

TruScan RM Handheld Raman Analyzer for identification of cell culture media

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Summary

The Thermo Scientific™ TruScan™ RM handheld Raman spectrometer with Thermo Scientific™ TruTools™ software was evaluated for the identification of chemically defined (CD) powder cell culture media and feeds. Samples were collected and analyzed by the Thermo Fisher Scientific BioProduction Group (BPG). The data (Raman spectra) were analyzed and identification methods were developed using advanced statistical methods available in the TruTools™ software package. The specificity of TruScan RM handheld Raman technology combined with the use of advanced chemometrics available in the TruTools software proved to be a powerful combination for rapid identification of these products. The results demonstrated that the TruScan RM with TruTools was able to successfully confirm the identity of 13 common cell culture media and feeds.

Overview

Cell culture media, a primary material used in bioproduction, is a complex mixture often composed of amino acids, carbohydrates, vitamins, minerals, and salts. This complexity combined with the compositional similarities between different media make identity confirmation an analytical challenge. Due to increased regulatory pressure in the biopharmaceutical industry to confirm identity of all production materials, methods for cell

culture media identification must be developed. Various compendia provide identification methods for common, single component material; but there is little guidance for complex formulations such as cell culture media.

Current methods for cell culture media identification such as HPLC for quantification of amino acids, vitamins, etc. are costly (requiring specialists and long analysis times) and may require formulation information which may not be available. The BioProduction Group (BPG) and Analytical Instruments Group (AIG) have teamed up to provide our customers with a more efficient solution. We evaluated the TruScan RM Handheld Raman Analyzer to be used for identity confirmation of chemically defined media and feed powders. Raman spectroscopy has several advantages over the traditional methods: (1) there is no sample prep; (2) analysis is fast, easy, and has good specificity; (3) the instrument is portable and so allows for analysis in manufacturing areas; and (4) formulation information is not required

Raman spectroscopy

Raman spectroscopy is a laser based vibrational spectroscopy method. The workflow specific to the TruScanTM RM is shown in Figure 1. The powdered sample is transferred into a glass vial and capped. The vial is then inserted into the handheld analyzer vial

TruScan RM handheld analyzer.

holder. The identification method is chosen by selecting the material name on the instrument screen and analysis begins. The instrument focuses the laser beam on the sample and a Raman spectrum is recorded by measuring the wavelength shifted light resulting from Raman scattering. The sample spectrum is compared against a qualified reference spectrum of the same material that is stored on the device. The result is a “Pass” or “Fail” based on the comparison of the sample spectrum with the reference spectrum. The entire process takes only minutes to complete.

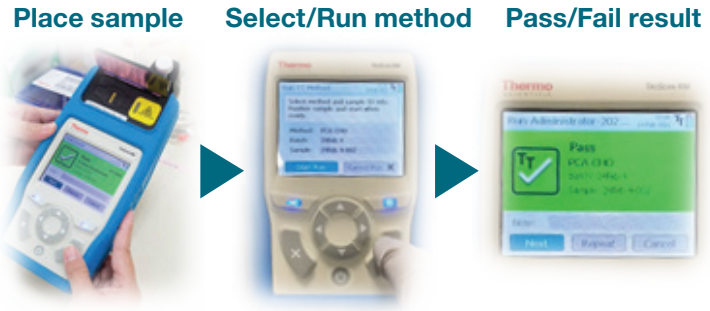

Figure 1. Workflow for Raman spectroscopy analysis.

A Raman spectrum of a cell culture medium is shown in Figure 2. The Raman spectrum is characteristic of the chemical makeup of the sample. More specifically, the Raman peaks correspond to vibrational modes of the molecules present in the sample. The Raman spectrum of cell culture media is complex with many overlapping peaks since the media is composed of a large number of components. Despite the complexity, the chemical differences between different media or feed products may result in a different pattern of Raman peaks in the spectra. The Raman spectrum could therefore serve as a chemical fingerprint that another sample spectrum could be compared with for identification confirmation.

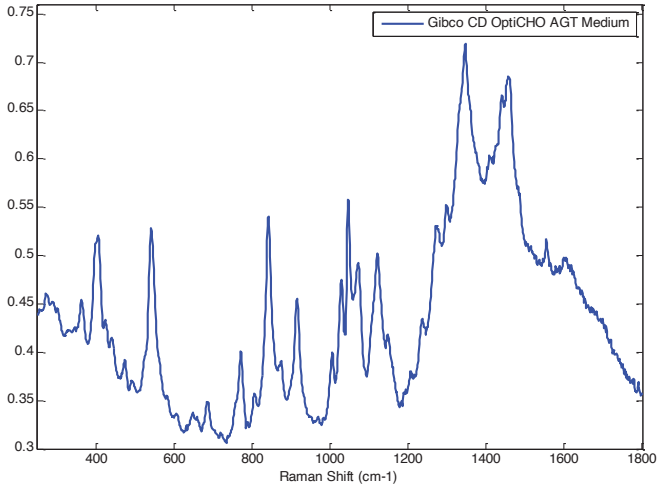

Figure 2. Raman spectrum of cell culture medium.

Experimental

The experiment was designed to determine if the TruScan RM with TruTools was able to differentiate different types of powdered media and feed products utilizing their unique Raman spectra. Four different cell culture media and nine different feeds were chosen based on their chemical composition. Some formulations are chemically similar, while others have a distinctive chemical composition. This range of cell culture media products should result in some obvious spectral differences and also should challenge the limits of the Raman technology. Raman spectra were collected for multiple lots (3-5) of each product. Three measurements were performed for each lot. This experimental

design allowed for a comprehensive comparison of spectral variation resulting from different scans, lots, and products.

Data acquisition

The laser power, detector exposure and number of coadditions of spectra for each measurement were optimized to give the best signal-to-noise and a reasonable measurement time. All products were measured using the optimized settings in Table 1. Overall, the spectra had very good quality for each product. The average spectrum for each product is shown in Figure 3 and an expanded region in Figure 4.

Laser power	250 mW
Detector exposure	2000 ms
Number of coadditions	8 per spectrum

Table 1. Optimized settings for TruScan RM data acquisition of powder media and feeds

Visual inspection of spectra

The expanded region in Figure 4 highlights the complexity of the Raman spectra. The spectral complexity was expected and reflects the rich diversity of components present in cell culture media as well as the vast number of components that the Raman can detect in a single scan. A successful identification method requires unique and reproducible spectral differences between products.

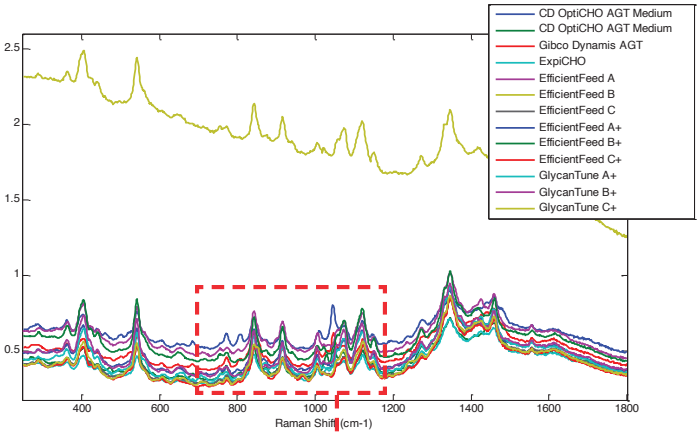

Figure 3. Average Raman spectra for various CD powder cell culture media and feeds.

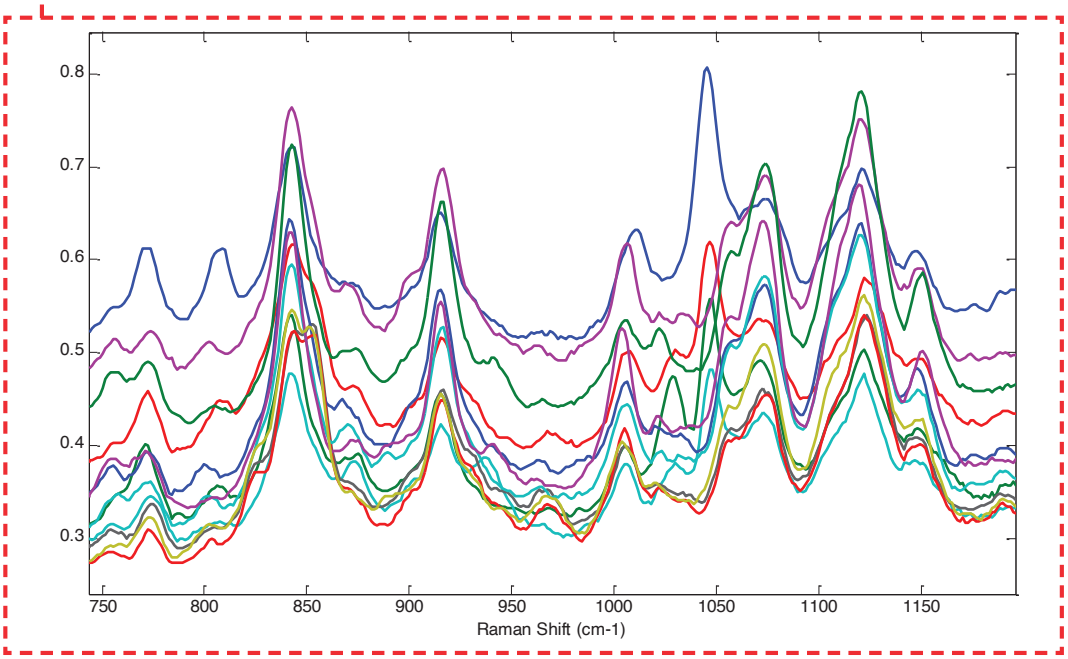

Figure 4. Zoomed region of Figure 3.

As anticipated, the Raman spectra of some products when compared to others were very similar and some were very different. Figure 5 is a plot of the average Raman spectra (baseline corrected) of four different CD media. There are subtle differences in the spectra of each product that may be used to differentiate one

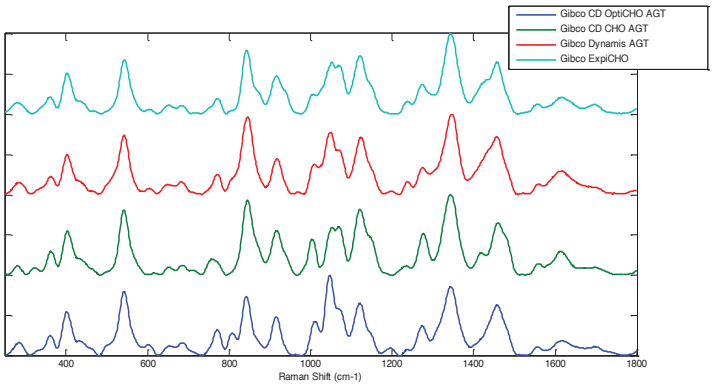

Figure 5. Average Raman spectra of various CD powder media.

medium from another. The Raman spectra of four CD powder feeds are plotted in Figure 6. Visually, there are not many differences in the spectra and so it will be more challenging to differentiate them by Raman spectroscopy.

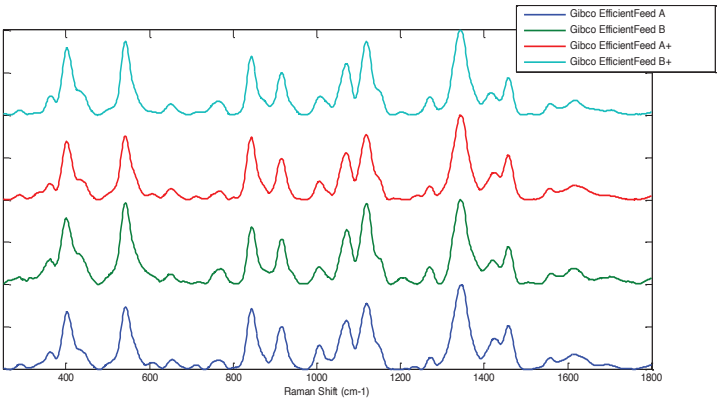

Figure 6. Average Raman spectra for various CD powder feeds.

The default p-value method typically used with the TruScan is a general-purpose classification method ideal for differentiating materials with obvious spectral differences. A different approach is required for these products because the spectral differences between products are minor. As an alternative to the p-value method, TruTools methods can be custom built to utilize small spectral differences to differentiate products and therefore enhance method selectivity.

Revealing and understanding spectral variations is necessary for robust TruTools methods. Therefore, spectral differences between products and lots were closely examined. Spectral differences between Gibco™ CD OptiCHO AGT™ and GibcoTM EfficientFeed C are discussed here as an example. Figure 7 plots the baseline corrected Raman spectra for three different lots of Gibco™ CD OptiCHO AGT™ measured in triplicate. The plot shows three traces for each lot that have relatively low variability within the same lot. This indicates that instrument variance and content uniformity is not a significant source of spectral variability for this product. However, spectral variation between lots was observed for this sample especially in the region 1000-1100 cm^{-1}. This spectral variation is due to the known polymorphism of HEPES present in the product. The differences in crystallinity result in different vibrational frequencies and so produce a reproducibly different Raman spectrum for each lot. Other Raman peaks not associated with HEPES are unaffected. The TruTools methods must be trained to learn these sources of variation. For this reason, it is important to collect and analyze as many lots as possible so that all relevant spectral variation is included in the training data. The Raman spectra for EfficientFeed C are plotted in Figure 8 for comparison with OptiCHO. The spectral variation for EfficientFeed C is relatively small and the overall spectral profile is visually different than that of OptiCHO. EfficientFeed C can then be easily differentiated from OptiCHO with Raman spectroscopy.

TruTools

Chemometric methods available in TruTools were used to evaluate the spectral variation for all products to determine if each product could be identified by its unique Raman spectrum. Principal component analysis (PCA) works by reducing dataset dimensionality in an interpretable way, such that most of the information in the data is preserved. The PCA scores plot for all products analyzed is shown in Figure 9a. Each point on the PCA plot corresponds to an individual Raman spectrum for a given product. The center of the 3D plot (0,0,0) represents the average Raman spectrum for all products (i.e. the average of all spectra in Figure 3). Each axis is a specific principal component corresponding to a deviation from that average. A PC axis may be related to the presence or absence of a certain media component or other sources of spectral variation such as a polymorphism or moisture effects. While possible, the exact source of spectral variability was not determined for this study.

The success of a PCA method for identification can be determined by assessing the PCA plot. A successful PCA method will have points for each product grouped together with all groups spaced far apart. Product groups are visualized as the colored spheres in Figure 9a. The size of the group is an indication of the extent of spectral variability for that product. Product-to-product spectral differences, which are important for identification purposes are indicated by the distance between the product groups.

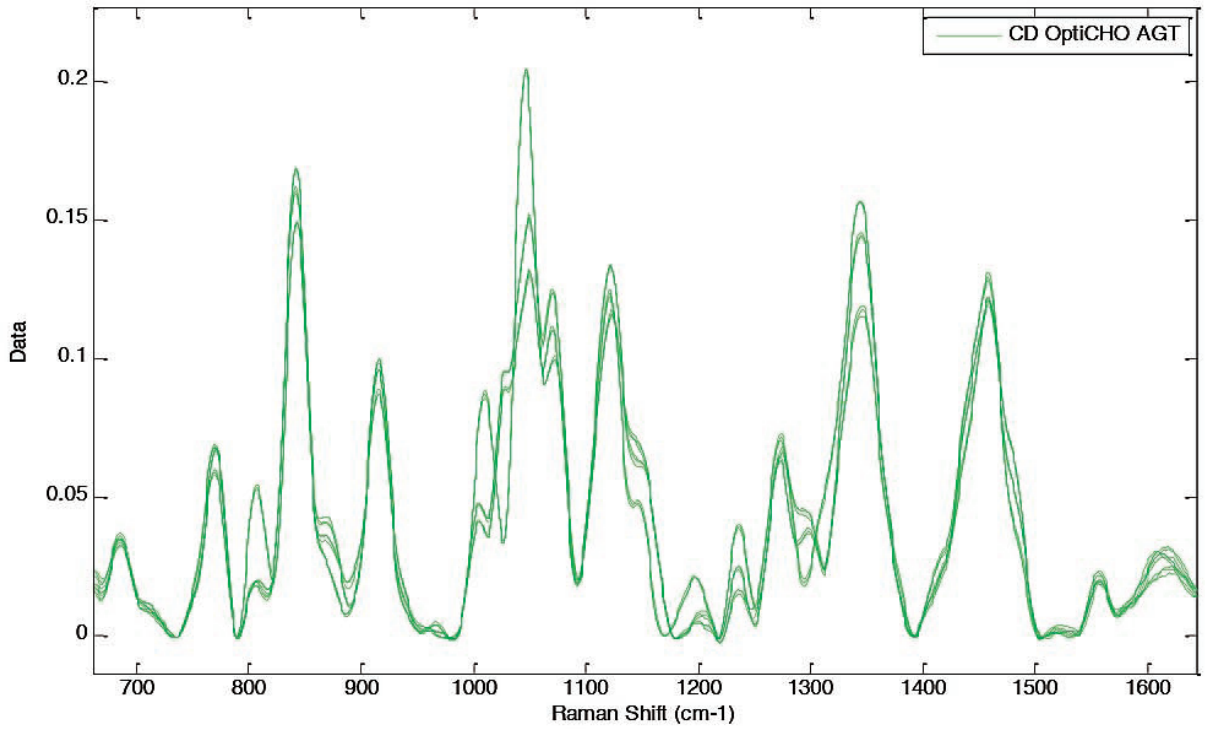

Figure 7. Raman spectra for three different lots of Gibco™ CD OptiCHO AGT™. Each lot was measured in triplicate.

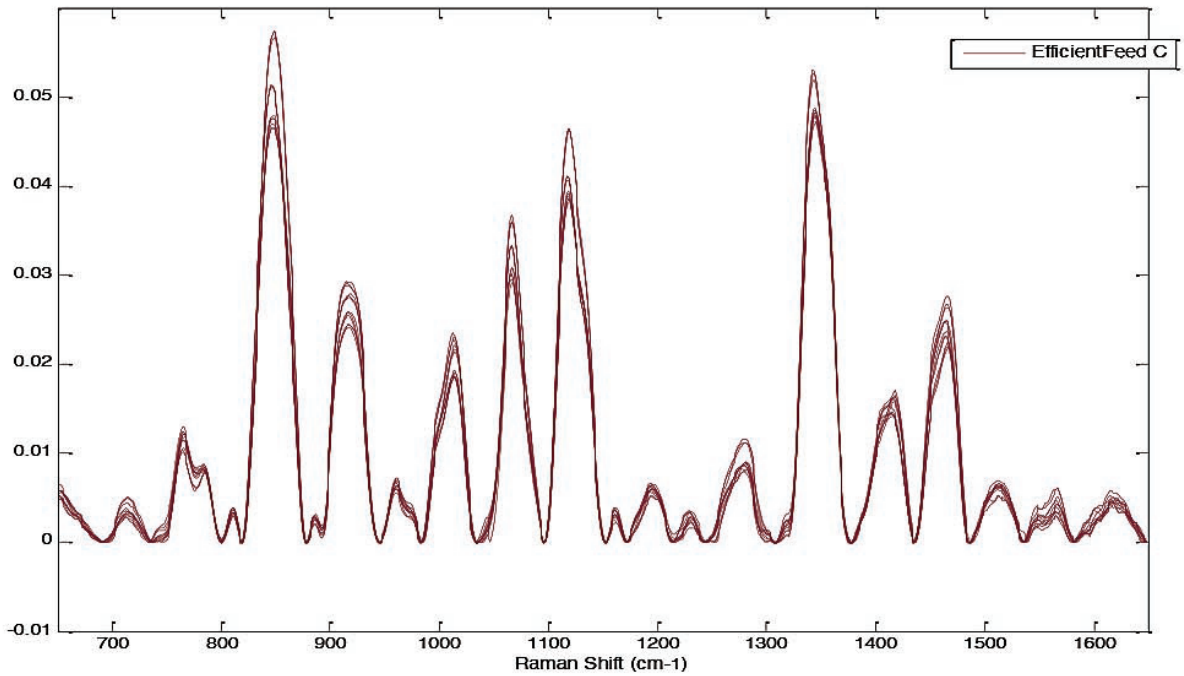

Figure 8. Raman spectra for different lots of Gibco™ EfficientFeed C. Each lot was measured in triplicate.

The Gibco Dynamis AGT™ samples (solid blue triangles in the upper-center) of Figure 9a are all grouped together and the group is spaced far from the other groups. This shows that the spectral differences are unique for Dynamis and reproducible, therefore, the TruScan with PCA may be used to confirm its identity. Additionally, there are four other clusters of product groups in the PCA plot. The products in each cluster are chemically similar and so have similar Raman spectra. The PCA plot was replotted based on these clusters as shown in Figure 9b. Note that the measurement points in Figure 9a are in the same position as in Figure 9b and are only regrouped based on proximity in the plot. Specifically of the media products: CD CHO, CD OptiCHO, and ExpiCHO had similar Raman spectra and are a single cluster. Additionally, the A feeds, B feeds, and C feeds all had their own cluster. That is, the A feeds had different Raman spectra than the B and C feeds, but the three A feeds had similar Raman spectra.

A summary of the PCA groupings is shown in Figure 10. The PCA method can uniquely identify the Dynamis AGT media product. Four other sets of three products have very similar spectra and require an additional test to differentiate them. This 2nd test may be an orthogonal analytical method or a more stringent chemometric method. In this study we used a more stringent chemometric method, PLS-DA.

The subsequent PLS-DA method was more stringent because it was optimized to discriminate between only three products rather than all products like the PCA model. PLS-DA is similar to PCA and are both multivariate data reduction methods. The most significant difference is that PCA is an unsupervised method and PLS-DA is a supervised method. More specifically for this case, PLS-DA includes the product name classification information in the iterative data reduction calculation while PCA intentionally ignores this information. A separate PLS-DA model was developed for each cluster of three products which had ambiguity in the PCA model.

The output for the group of C feeds PLS-DA calculation is shown in Figure 11. The plot in the upper left is the Q Residuals on the y-axis and the Hotelling T^2 on the x-axis for each sample. The Q Residuals (y-axis) relates to the amount of variability in the spectra not explained by the model. The Hotelling T^2 quantifies how “different” each sample is from the average sample while only considering the spectral variations defined in the model. The samples are grouped together and there are no significant outliers. This indicates that the spectra for all the samples are explained well by the PLS-DA model and that not one product or sample varies greatly from any other.

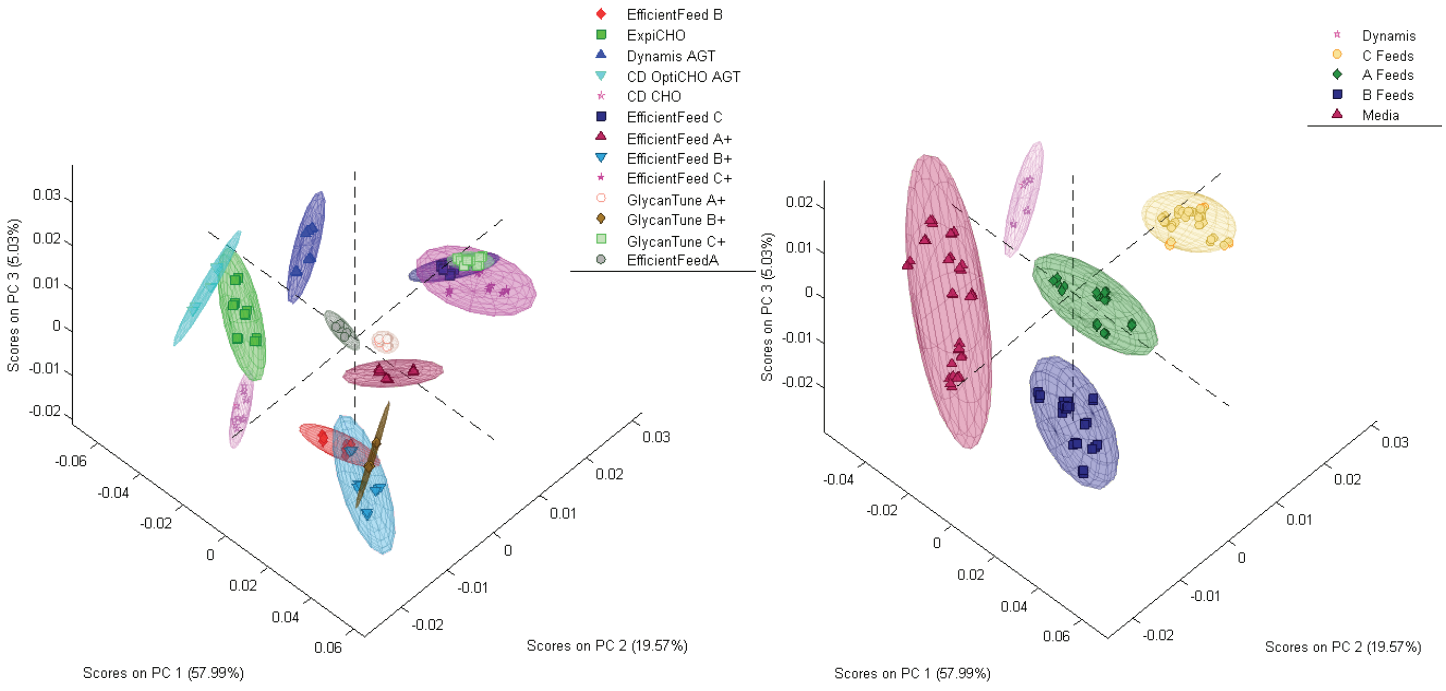

Figure 9. PCA scores plot for Raman spectra of 13 different CD powder media and feeds.
Figure 9a (grouped individually) Figure 9b (grouped in clusters).

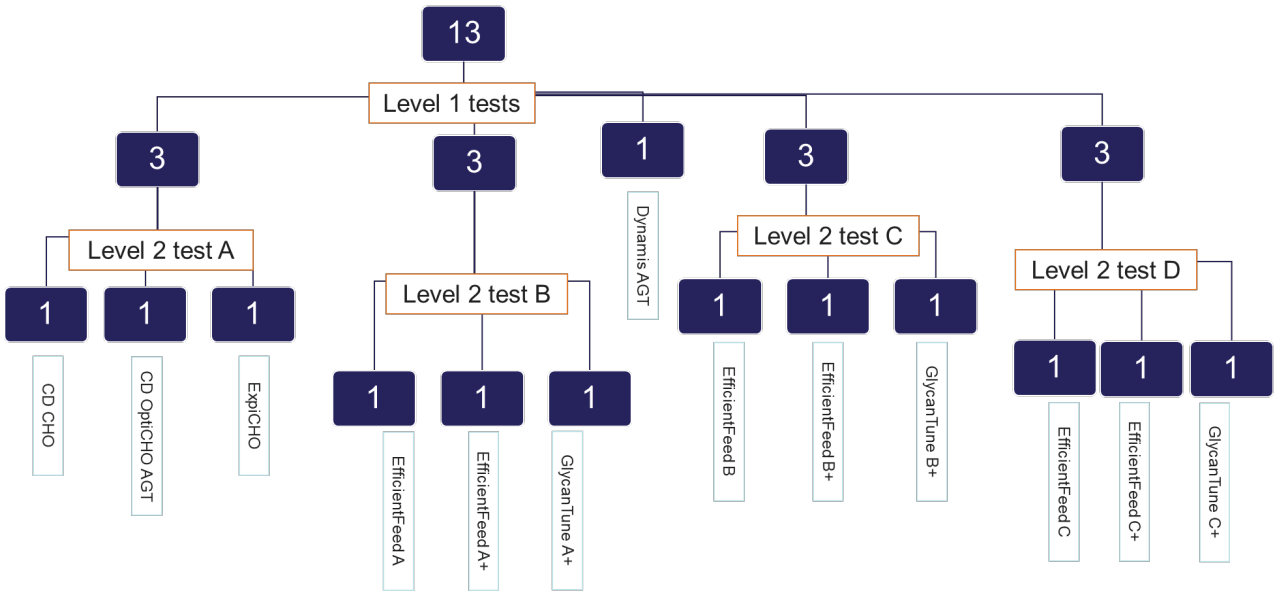

Figure 10. 2-level testing diagram based on groupings of products from PCA method.

The other three plots show the cross-validation (CV) identity predictions (y-axis) for all 3 products (EfficientFeed C is upper-right, EfficientFeed C+ is bottom left, and GlycanTune C+ is bottom right). The CV identity predictions are beyond the accepted threshold (upper dotted line) for each product. Additionally, the identity predictions are below the threshold for negative testing. For example, for EfficientFeed C (upper right), if the model input is the Raman spectrum of an EfficientFeed C sample then it has a relatively high probability of being identified as EfficientFeed C. If the model input is the Raman spectrum of an EfficientFeed C+ or GlycanTune C+ sample, then it has a relatively low probability of being identified as EfficientFeed C. These together show that the method can differentiate one product from the other two different C-type feeds.

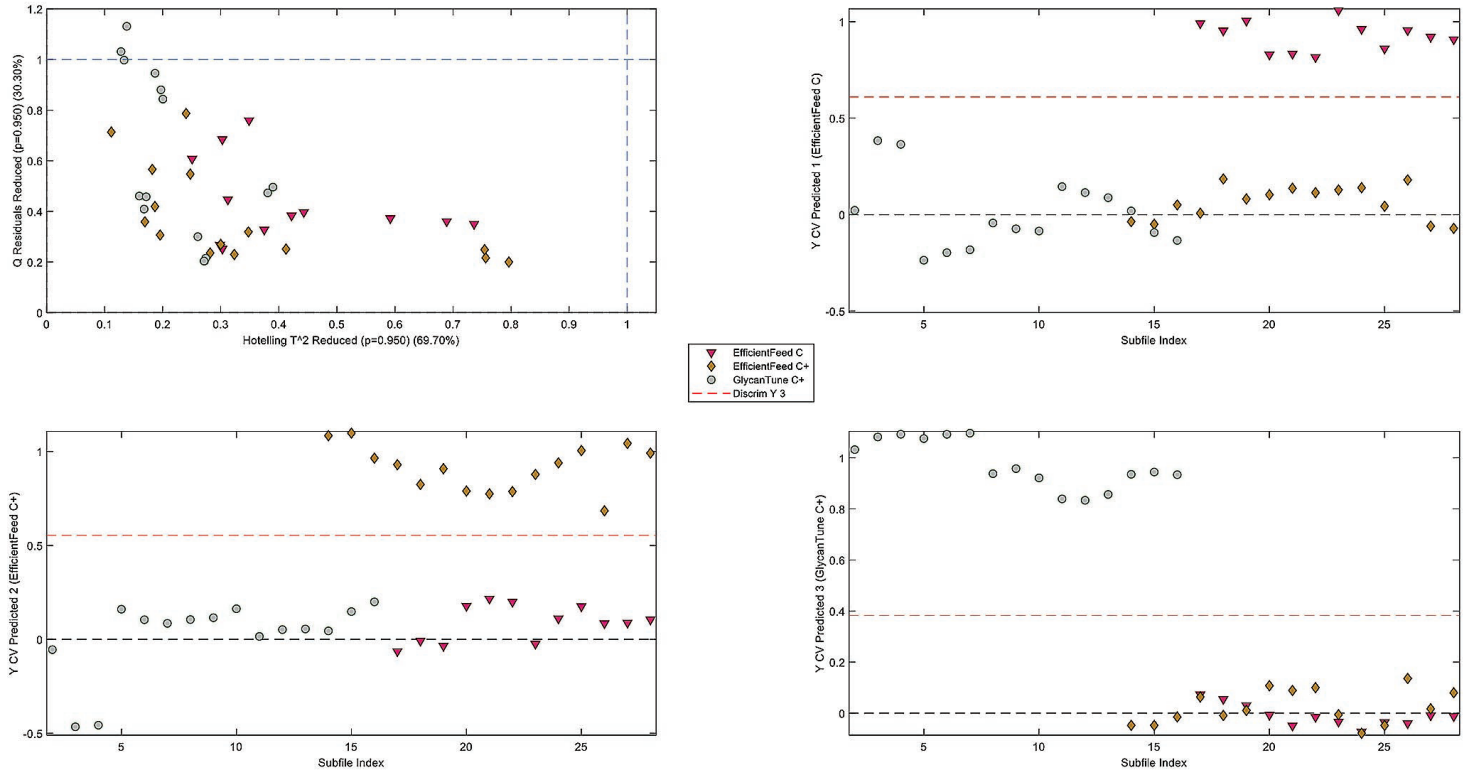

Figure 11. PLS-DA results for the group of C feeds.

Conclusions

The results of this study show that all 13 media and feeds in this study could be identified using a combination of PCA and PLS-DA. The TruScan RM with TruTools can be a powerful tool for identity confirmation of powder cell culture media and feeds. Currently, this PCA/PLS-DA combination approach could only be manually realized in TruTools. In the future release of TruTools, this workflow will be fully automated.

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