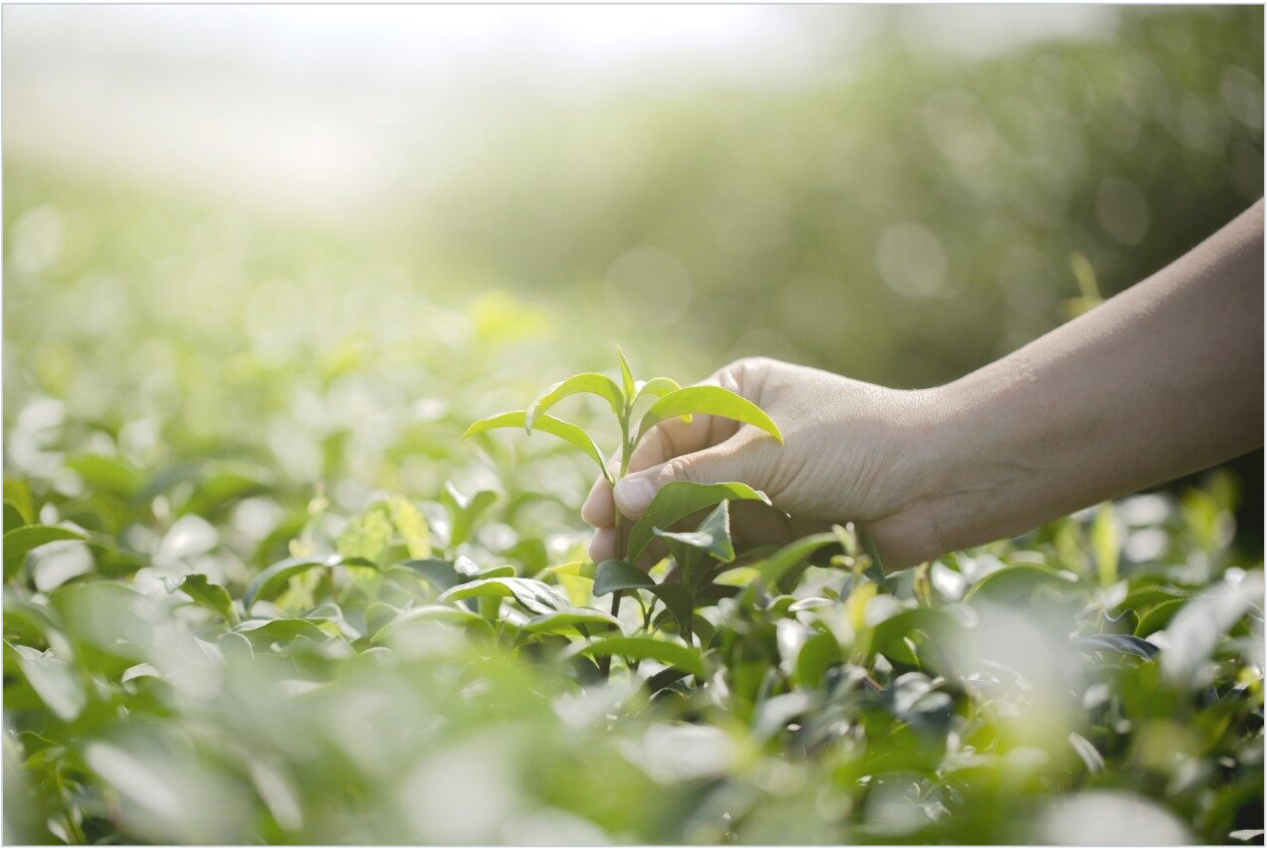
Application Note

Simple Differentiation of Black Tea Samples using a Chemometric Approach with MarkerLynx XS Application Manager

Antonietta Gledhill, Diana Uría

Waters Corporation



Abstract

This application note demonstrates to identify intra- and inter-class differences between black tea samples using UPLC-MS and a multivariate approach (MVA) with chemometrics-based software.

Introduction

Extensive testing of food and beverages is required in manufacturing, ranging from testing for contaminants (accidental or deliberate), monitoring and developing the composition, to testing end product for taste and flavor to ensure quality and consistency. Recent food safety scares have highlighted the importance of effective QC for both protecting consumers and minimizing the business risks associated with a product recall or failure.

Today's food manufacturers must meet the challenges presented by growing consumer demands within the food and beverage industry. This competitive environment adds to the already increasing need for simple, repeatable, and reliable testing techniques that can be used consistently, routinely, and with confidence in results.

One issue is the sourcing of raw materials that can include products from diverse geographies. Ensuring the consistency of supply and product composition is essential in maintaining product safety and quality. Many manufacturers are seeking to profile, or 'fingerprint' raw materials by using rapid and powerful analytical techniques capable of detecting contaminants and impurities before materials are processed.

As the world's black tea production is projected to increase to 2.4 million tons in 2010, issues with tea manufacturing processes that could lead to a poor quality product and negatively impact the consumer's experience need to be addressed.

Traditional approaches to the analysis of tea samples include monitoring differences within key compounds, or training people to recognize differences in specific ratios of compounds; however both processes can be extremely time-consuming and sometimes inaccurate.

The use of chemometrics can provide the analyst with more information than traditional techniques. The automation of this process will also relieve some of the demands placed on a trained analyst and can help reduce the occurrence of manual errors.

The following method describes the application of UPLC and single quadrupole mass detection for the analysis of commercially available black tea samples; one sample from an economy brand and three samples from premium brands. Overall differences were assessed via chemometric analysis using MarkerLynx XS Application Manager Software.

Experimental

Sample Preparation

- Remove the tea leaves from their bags
- Weigh 100 mg of leaves and add 100 mL of hot water
- Allow to infuse for at least three minutes
- Decant 1 mL of water and 0.45 µm filter

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 100 mm, 1.7 µm

A simple gradient of increasing percentage of acidified acetonitrile was used for the analysis. The run time was 7.5 minutes.

MS Conditions

MS system:	Waters ACQUITY SQD (SQ Detector)
Ionization Mode:	ESI negative and positive

ACQUITY SQD.

Acquisition and Processing Methods

Data were acquired using Waters MassLynx Software, v. 4.1. Thes data were processed using MarkerLynx XS Application Manager. This software package is designed to interpret multivariate data, such as the complex data sets obtained from mass spectrometers.

Results and Discussion

The method was developed using the ACQUITY UPLC System to enable rapid run times, as samples were looked at in duplicate to ensure reproducible retention times.

The SQ Detector simultaneously analyzed each sample in ESI positive and negative modes to better investigate the compounds present in the tea samples. The mass spectral information was then processed within MarkerLynx XS.

A generic gradient was used for the chromatographic conditions as the primary objective was to use retention time and the associated *m/z* value within MarkerLynx XS and allow the software to interpret the data and determine any trends observed.

MarkerLynx XS Software takes the *m/z* and retention time associated with it and populates them into a table. This produces a fingerprint for each sample analyzed that is represented in relation to the other samples by principal component analysis (PCA). MarkerLynx XS provides users with a full suite of advanced statistical tools, including partial least squares discriminate analysis (PLS-DA) and orthogonal partial least squares (OPLS), transforming data into information.

- PLS-DA is performed in order to sharpen the separation between groups of observations. It allows groups to be predefined and therefore does not allow for other response variables — all measured variables play the same role with respect to the class assignment.
- OPLS is a modification of the usual PLS model that allows the user to compare two groups of samples, for example, good products versus non-conforming products.

These advanced statistical tools, along with a complete graphical display suite as shown in Figure 1, allow researchers to visualize their data and extract more meaningful information from their experimental results.

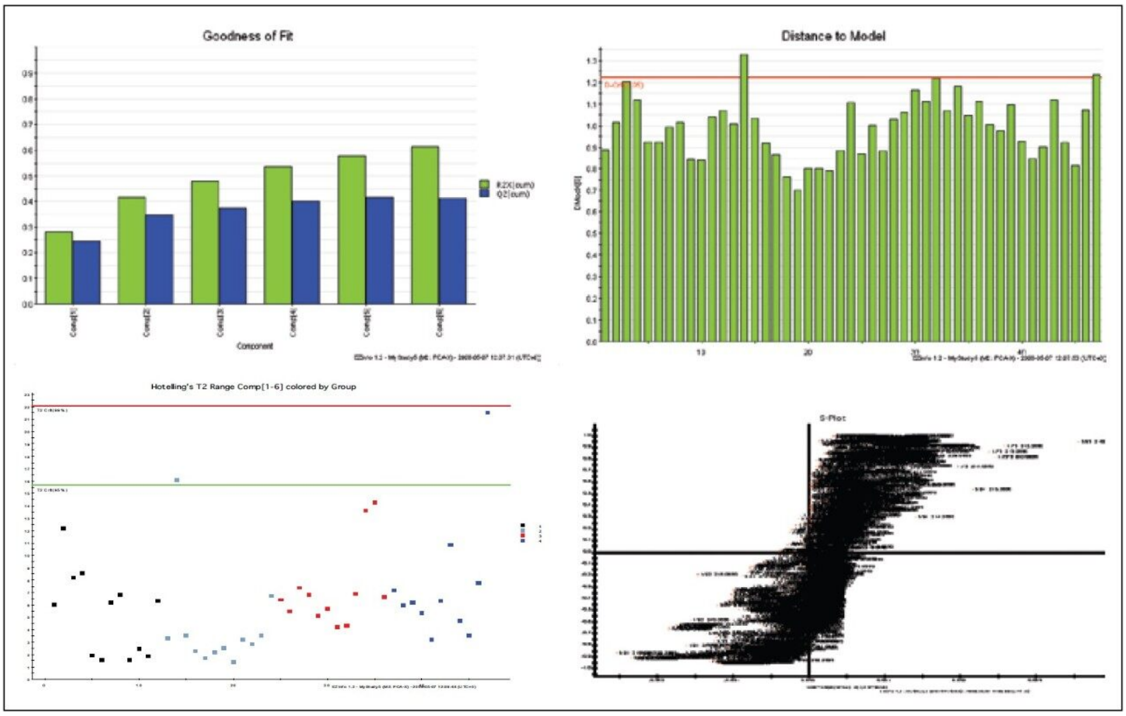


Figure 1. A suite of statistical plots that is available within MarkerLynx XS Application Manager.

This additional functionality allows sample differentiation, including batch-to-batch comparisons, to be performed quickly and easily.

Two models were selected to view the tea sample data: the first was the PCA model and the second the PLS-DA model.

Figure 2 shows the scores plot — differences between the samples — using the PCA model. Three of the tea groups cluster together (the premium samples), suggesting they are similar, and the other tea group (the economy sample) is separated from the main cluster.

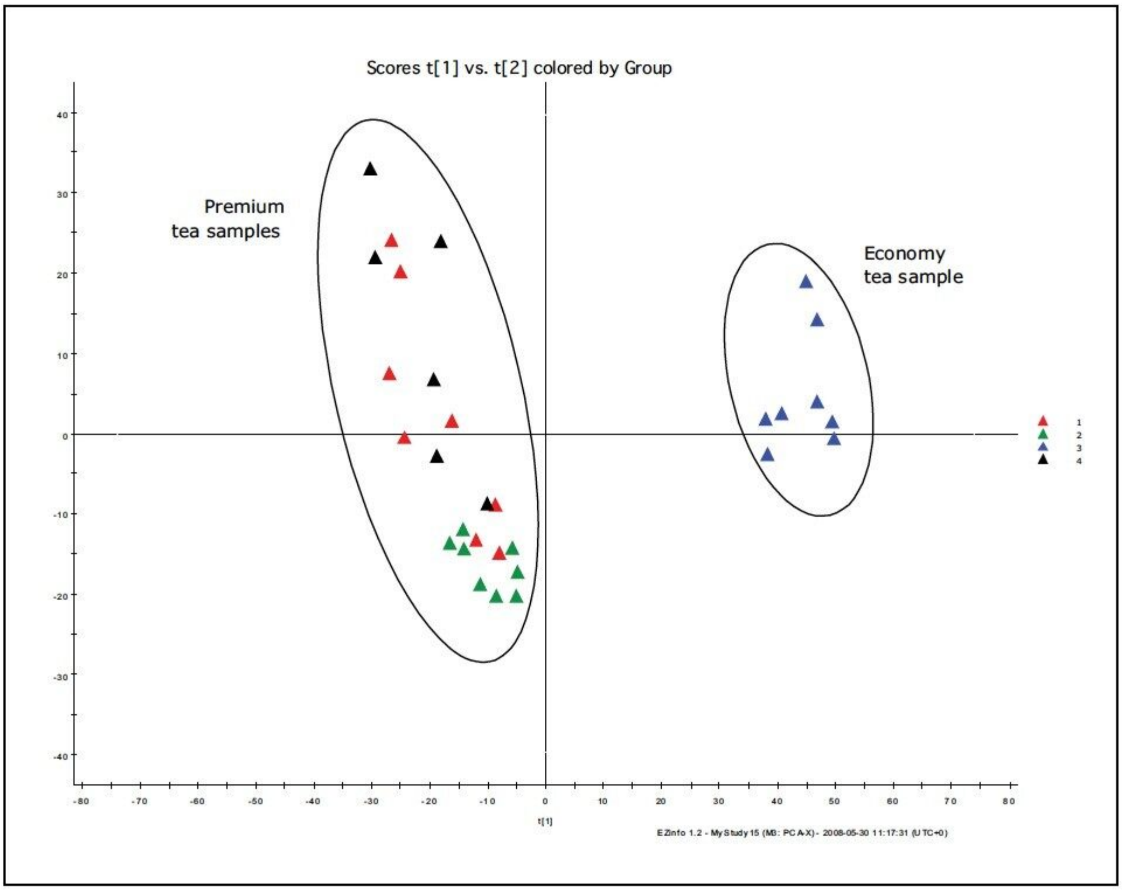


Figure 2. Scores plot using a PCA model.

This difference can also be seen in Figure 3, the three-dimensional (3-D) viewer of the PCA model. In this example, Tea 3 (economy brand) is grouped away from the other three teas. Rotating this model does not separate premium brand Teas 1, 2, and 4 away from one another.

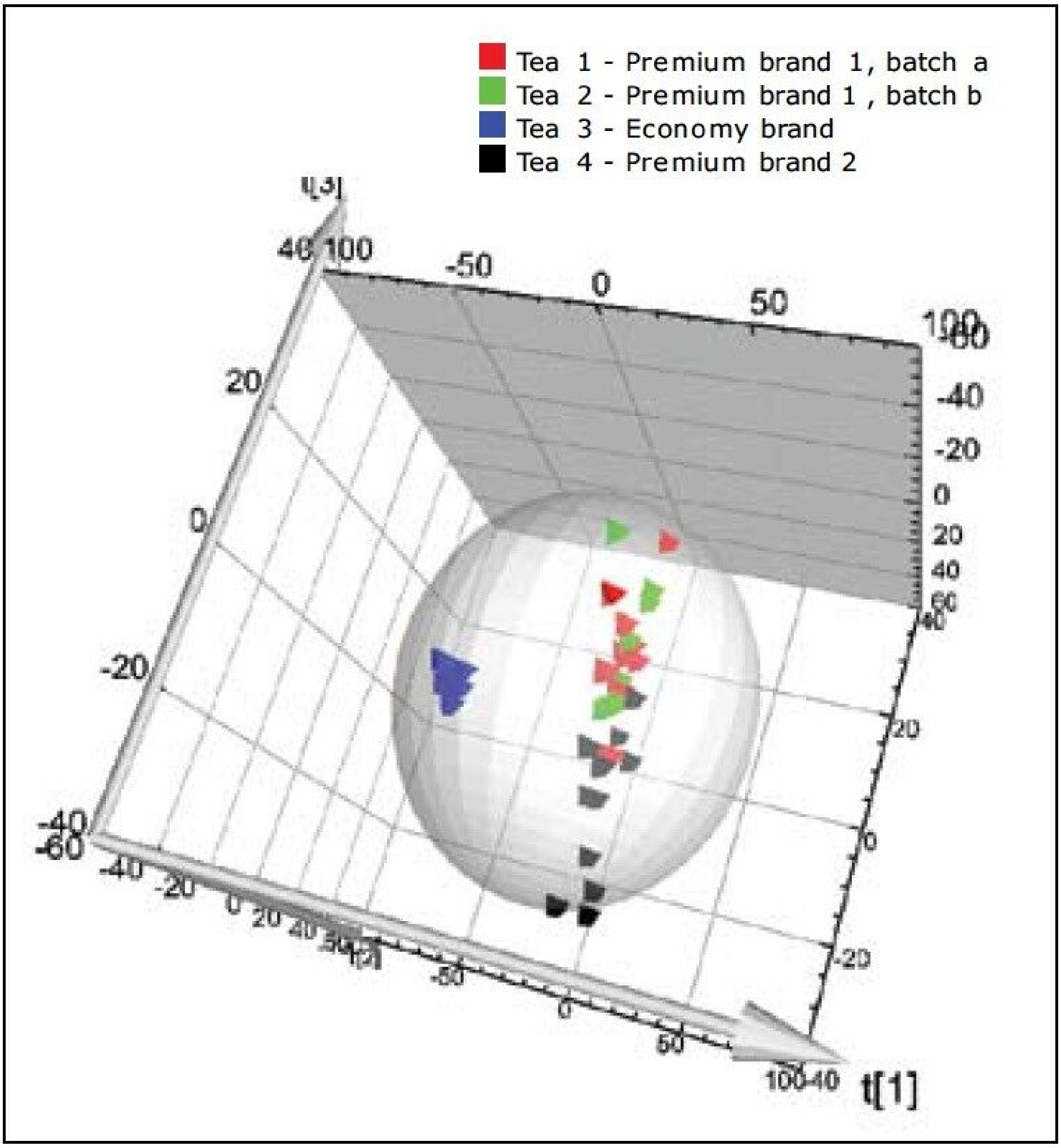


Figure 3. 3-D viewer from MarkerLynx XS using the PCA model.

Using the 3-D viewer from the PCA and PLS-DA models, it is possible to see trends and patterns that are not as apparent in a two-dimensional view. The 3-D view becomes an especially powerful tool when the history of samples is known, for example if the manufacturing process is known to have been successful.

As seen with the PLS-DA data, Figure 5 not only shows the main clustering seen using the PCA model, but a slight difference can now be seen between Teas 1 and 2 (same premium brand but different batches) and Tea 4 (different premium brand).

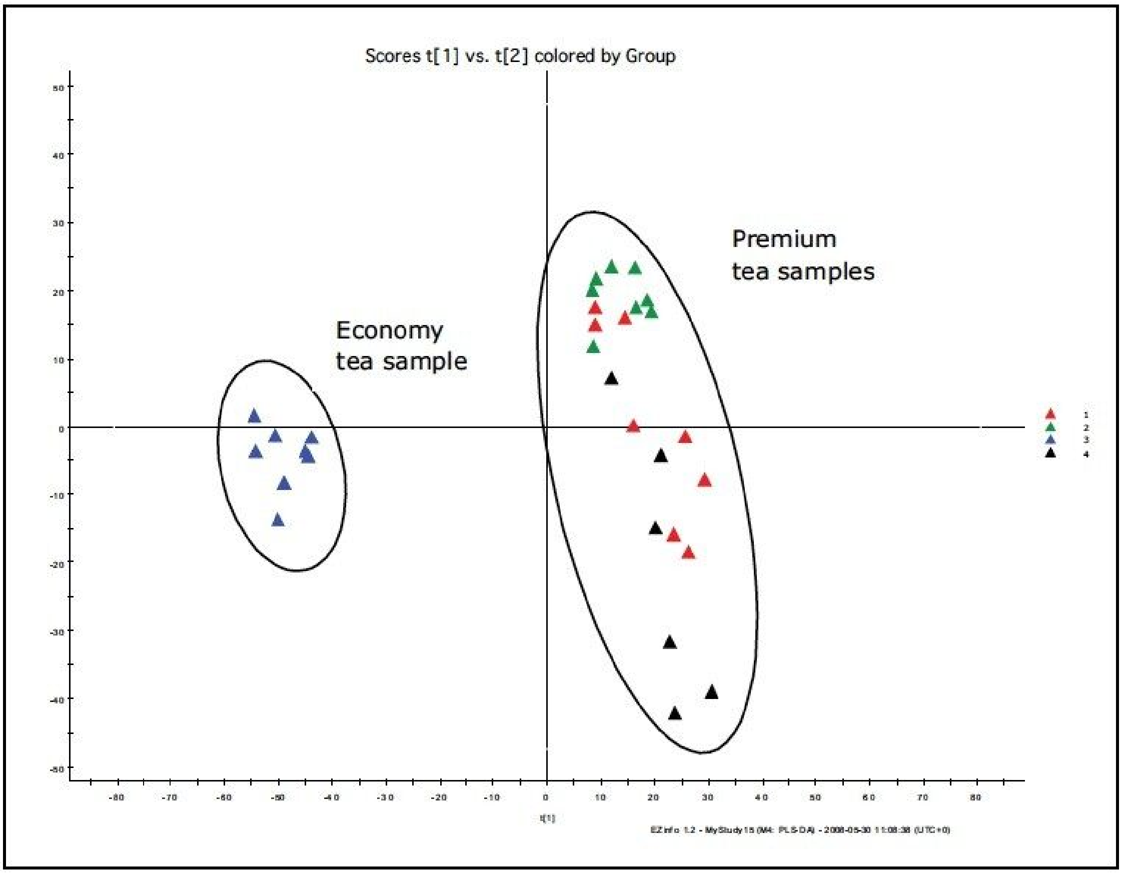


Figure 4. PLS-DA model of the four teas.

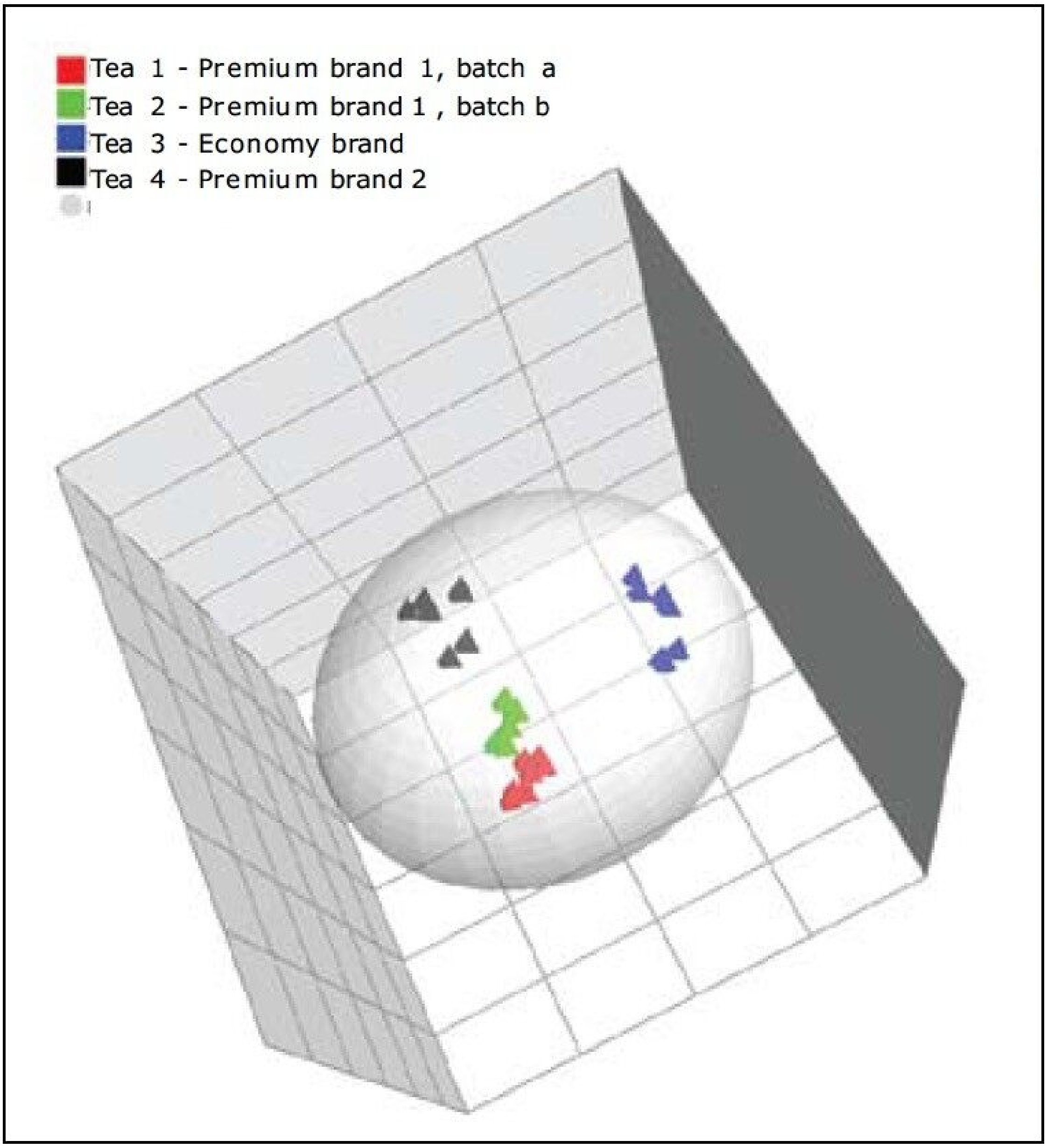


Figure 5. 3-D scatter plot of PLS-DA data for the four tea samples.

The 3-D PLS-DA model, as shown in Figure 5, accentuates the degree of separation seen for all four tea samples. The main factor to be considered when interpreting the data from a PLS-DA model is that the results need to be calibrated, by running samples notused in the model and seeing whether they fit correctly into the clusters created for that sample group.

Conclusion

The analysis of four different tea samples was performed using ACQUITY UPLC with the SQ Detector and MarkerLynx XS Application Manager to interpret the data.

Using this UPLC-MS system solution, it was possible to identify the intra- and inter-class differences between all tea samples; one economy brand and two premium brands, where two of the samples were the same brand, but from different manufacturing batches. Clear cluster grouping was achieved, accentuated by the 3-D view of the PLS-DA model data.

The power of UPLC enables rapid analysis times, which allows many samples to be compared in a very short amount of time. In addition, retention times are reproducible from sample to sample which is critical when looking at this type of comparison technique, as it is sensitive to changes between chromatograms.

The SQ Detector’s rapid polarity switching enables information from both positive and negative electrospray to be acquired and interrogated for comprehensive results.

This information-rich dataset was then processed using MarkerLynx XS Application Manager. This software is a multivariate analytical tool that identifies differences between samples using PCA; and differences between sample groups using PLS-DA. This fully automates the data analysis process, producing accurate, consistent results in a short time period.

The Waters UPLC-MS solution provides:

ACQUITY UPLC System for high productivity

- Rapid throughput with improved resolution and sensitivity for enhanced sample differentiation capabilities
- Flexible solution for analysis of multiple compounds in complex matrices
- Improves laboratory efficiency
- Delivers excellent return on investment for QC laboratory

ACQUITY SQD for simplified mass detection

- Rapid separation and detection of multiple compounds
- Provides an easy-adoption route for MS to speed up method development and improve method performance in a QC environment

- IntelliStart technology reduces the burden of complicated instrument operation
- Small laboratory bench footprint

MarkerLynx XS Application Manager for automated data analysis

- Extracts relevant marker information and processes complex multivariate data from LC-MS analyses
- Via an interactive browser, performs data reduction and statistical analyses to identify characteristic markers of specific compounds within similar products, that can then be used to distinguish differences in the properties of final commodities.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

SQ Detector 2 <https://www.waters.com/134631584>

Progenesis QI <https://www.waters.com/134790652>

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