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應用手冊

Distinguishing Between Tea Varieties Using Multivariate Analysis of UPLC-MS Data

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Abstract

In this study, we investigate the analysis of tea samples using the Waters ACQUITY UltraPerformance LC System

and the Waters Micromass LCT Premier XE Mass Spectrometer, and subsequent multivariate analysis using the MarkerLynx Application Manager for MassLynx Software.

Introduction

More tea is consumed around the world than any beverage except water. Camellia sinensis is an evergreen native of China and gives rise to more than 3,000 varieties of tea worldwide, which can be roughly classified into six basic categories: white, green, oolong, black, pu-erh, and flavored. Additionally, herbal infusions are growing in popularity. As preferences are largely regionalized, the two most popular varieties are green (favored in Asian countries) and black (favored in the western countries). These varieties often contain differing concentrations of antioxidative polyphenols, as well as other constituents that are often promoted for their health benefits. Each tea is a complex mixture giving rise to its own unique flavor. Further, the concentration of the components varies between sample varieties. A simple comparison of one or two components from each tea will not suffice to fully differentiate between the varieties because there are hundreds of components to track. Hence, a multivariate statistical analysis approach is required to fully distinguish between them. In this study, we investigate the analysis of tea samples using the Waters ACQUITY UltraPerformance LC System and the Waters Micromass LCT Premier XE Mass Spectrometer, and subsequent multivariate analysis using the MarkerLynx Application Manager for MassLynx Software. The advantage of this approach is that UPLC-MS produces a unique spectrometric “fingerprint” for each tea sample, allowing for easy statistical separation between the varieties.

Experimental

Tea Samples

Ten tea varieties were selected for this study, five traditional teas and five herbal infusions (noncaffeinated herbal teas). The caffeinated teas were: Japanese Green Tea, Lotus Green Tea, Traditional English Tea, Earl Grey, and Chai Tea. The non-tea herbal infusions were: Tension Tamer Tea, Cranberry, Raspberry, and Elderflower Tea, Lemon and Ginger Tea, Black Current, Ginseng, and Vanilla Tea, and Chamomile Tea. The teas were brewed using hot water in a typical cup volume as per package directions and diluted 1:4 with distilled water before analysis. Each aliquot was allowed to sit in a refrigerator for one week to simulate iced tea.

LC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 100 mm, 1.7 µn
Flow rate:	0.600 mL/min
Mobile phase:	A: 0.1% formic acid in water, B: 0.1% formic a acetonitrile
Injection volume:	5 µL
Sample temp.:	10 °C
Column temp.:	40 °C
Gradient:	See Table 1

Step	*Time (min)*	*Flow Rate (mL/min)*	*%A*	*%B*	*Curve*
1	Initial	0.770	100	0	Initial
2	0.36	0.770	100	0	6
3	0.60	0.770	88	12	6
4	2.10	0.770	79	21	6
5	2.40	0.770	75	25	6
6	3.0	0.770	5	95	6

Table 1. Gradient table for a 3-minute separation. A proportional factor of 3.33 and 10 were applied to the run-time for the 10- and 30-minute separations, respectively. The column equilibration time was maintained at 6 column volumes for all analyses.

MS Conditions

MS system:	LCT Premier XE Mass Spectrometer
Ionization mode:	ESI+ and ESI–
Range:	50–850 *m/z*
Cone voltage:	70 V
Capillary voltage:	3200 V in positive ion mode and 2800 V in negative mode
Scan time:	0.3 s
Interscan delay:	0.1 s
Desolvation gas:	700 L/hr
Cone gas:	50 L/hr
Source temperature:	350 °C
LockSpray:	Leucine enkephalin at 30 µL/min
W-Optics mode:	Enabled
DRE:	Enabled

Results and Discussion

Qualitatively, differences between the tea samples could be discerned by visual inspection of the UPLC-MS chromatograms. If a simple comparison between two chromatograms was required, a peak-bypeak comparison would be sufficient to yield the retention time and *m/z* (RT-*m/z*) pairs for sample comparison. In this case, the data set under investigation consisted of multiple analyses of ten tea varieties (n>6). A manual chromatogram-

bychromatogram inspection would be very labor-intensive.

For this complex task, MarkerLynx was utilized to automatically classify tea varieties based on spectrometric data sets using Principal Components Analysis (PCA) and to generate a table consisting of RT-*m/z* pairs ranked in order of statistical significance. This process allows the analyst to easily distinguish one tea sample from all of the other varieties. Figure 1 illustrates MarkerLynx PCA results after processing the 5 caffeinated teas. All five tea types show tight clustering amongst their respective sample populations. Figure 2 demonstrates the data richness of the ESI- UPLC-MS data to be analyzed. As the caffeine ion peak intensity obscures the peak scaling in ESI+ mode making data comparison difficult, it was omitted. This further demonstrates why manual peak inspection would be difficult and laborious.

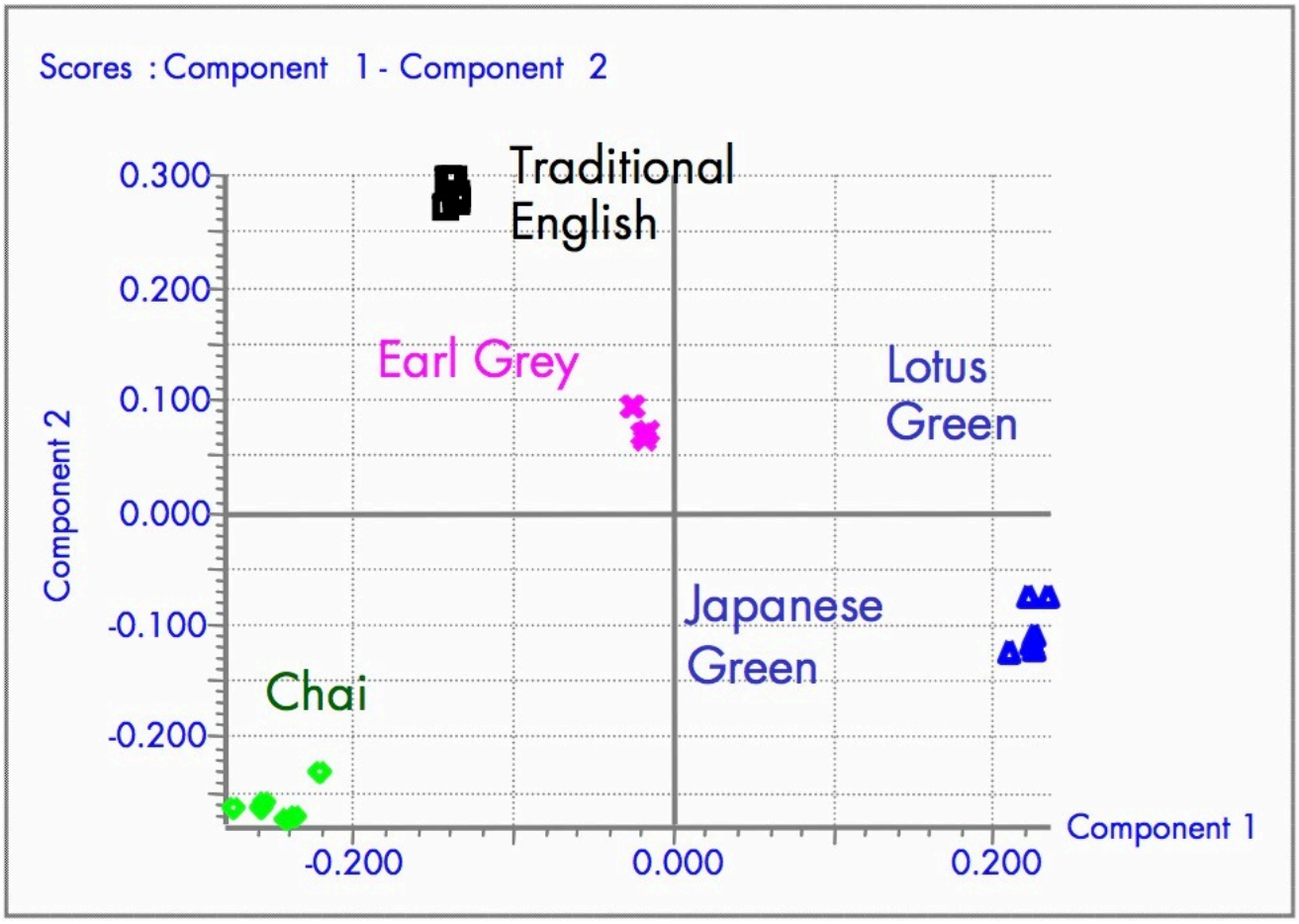

Figure 1. Scores plot obtained from MarkerLynx, illustrating group similarities between the five caffeinated tea types. The retention time and m/z ratio information for caffeine was removed.

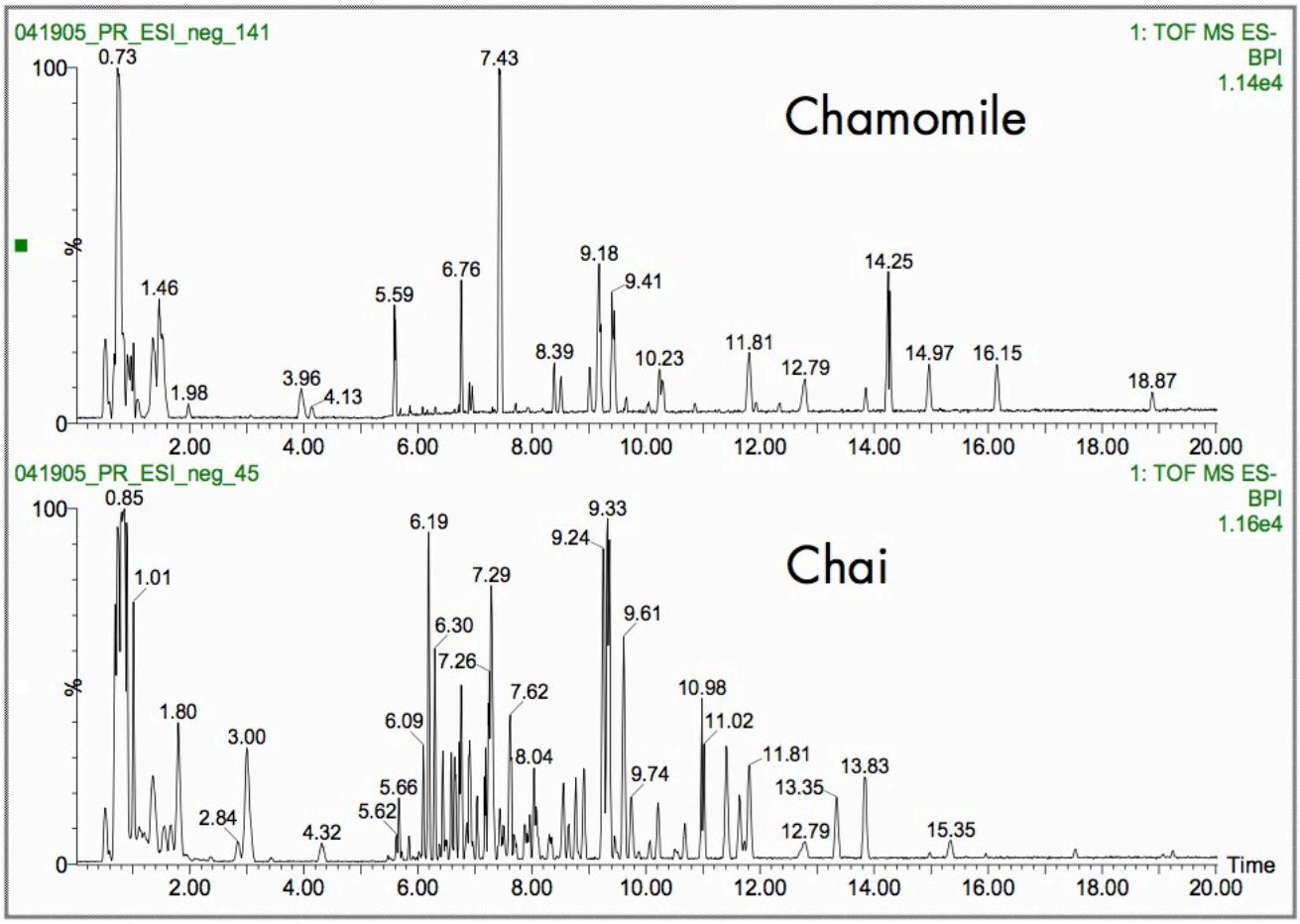

Figure 2. Comparison UPLC/oa-TOF ESI- chromatograms of Chamomile and Chai teas. Zoom-in perspective of a 30-minute analysis.

All of the MarkerLynx analyses discussed in this work were conducted on the ESI+ data because the resulting PCA separations were more distinct. The caffeine ion (M+H = 195.0882) was removed from all PCA statistics shown because it was the major feature in all caffeinated teas samples and tended to skew the results.

Analysis Time Selection

The data in Figure 2 shows a comparison between the herbal infusions Chamomile and Chai from a 30-minute UPLC-LCT Premier XE gradient separation, illustrating the qualitative differences. However, when analyzing a chemically-reactive system like freshly brewed tea using long LC separation times, detrimental chemical reactions could occur. This could result in qualitative and quantitative differences between samples analyzed at the beginning and end of the analytical run. To reduce or eliminate these side reactions, the extra resolving power and throughput of ACQUITY UPLC was harnessed in order to decrease analysis times while maintaining superb data quality.

When employing a fast 3-minute UPLC gradient separation, it is important to determine if there is any loss in data quality. Figure 3 shows the trend plots (sample vs. normalized ion intensity) for catechin gallate, one of the common components in tea, from three different separation run times: 3, 10, and 30 minutes. This data clearly demonstrates that there is no apparent loss of information by using the shorter 3-minute separation when compared to the longer 10- or 30-minute separations. The shorter UPLC analysis generates sufficient performance to locate group similarities and identify the components responsible without missing important analytes. Additionally, there is a substantial times savings by employing the shorter analysis time – approximately 3 hours for all 10 tea samples (6 replicates x 10 teas) using the 3-minute separation, while 30 hours is required to analyze the same samples using the 30-minute separation. (This calculation does not include the system QC test that was run every 8 injections.)

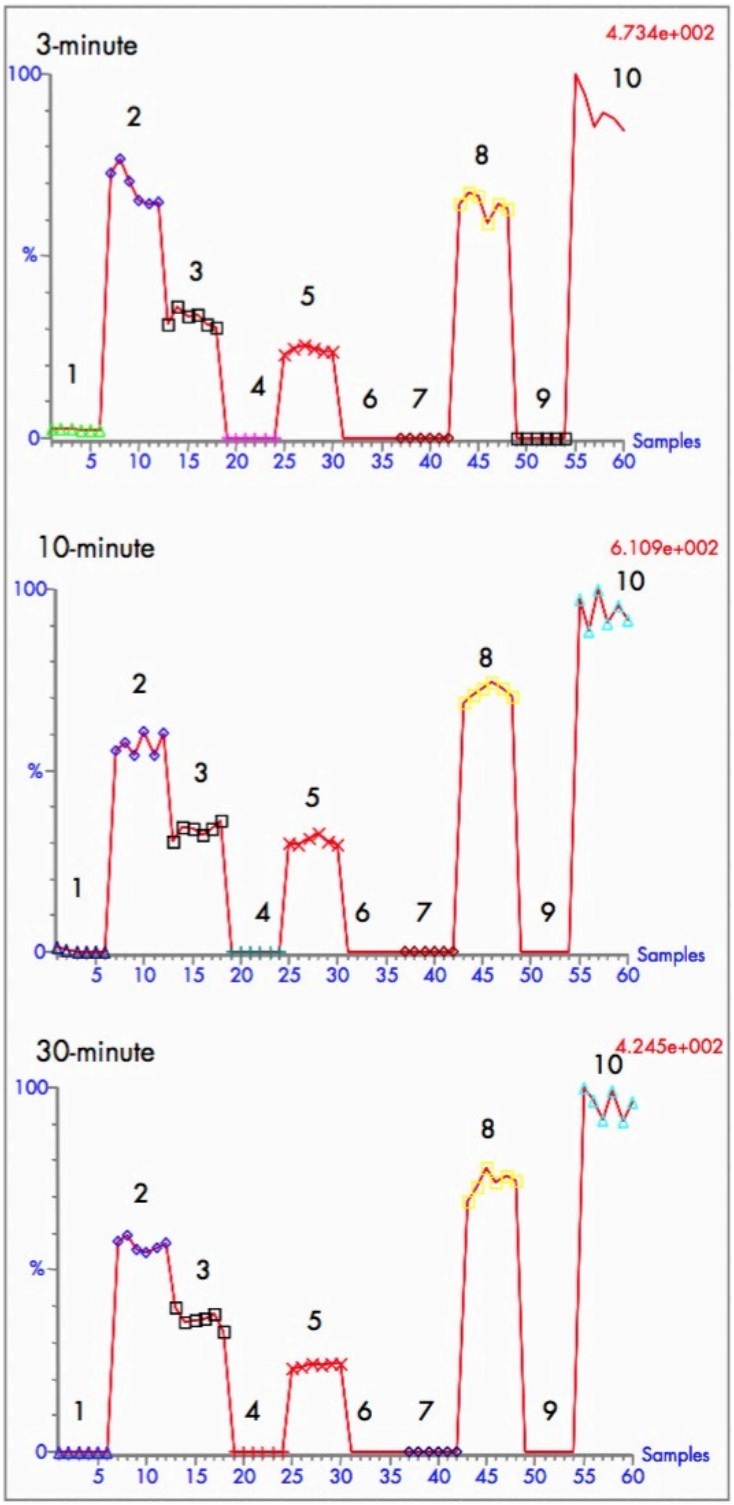

Figure 3. Trend plots (samples vs. ion intensity) demonstrating the fidelity of the ion intensity information for catechin gallate across three chromatographic run times. Tea samples: 1) Tension Tamer, (2) Japanese Green, (3) Chai, (4) Cranberry, Raspberry, and Elderflower, (5) Traditional English, (6) Lemon and Ginger, (7) Black Current, Ginseng, and Vanilla, (8) Earl Grey, (9) Chamomile, and (10) Lotus Green.

Multivariate Analysis using MarkerLynx The data in Figure 1 shows the classification of the five caffeinated teas investigated from a 3-minute ESI+ UPLC/MS data. The high analytical reproducibility of the system is demonstrated through the tight sample clustering of each individual tea variety. Further, performing statistical

analysis on the entire data set (caffeinated and herbal infusion varieties) yields a clear distinction between the two types, as illustrated in the PCA scores plot (Figure 4). The RT-*m/z* pair for the caffeine ion was removed for this statistical analysis.

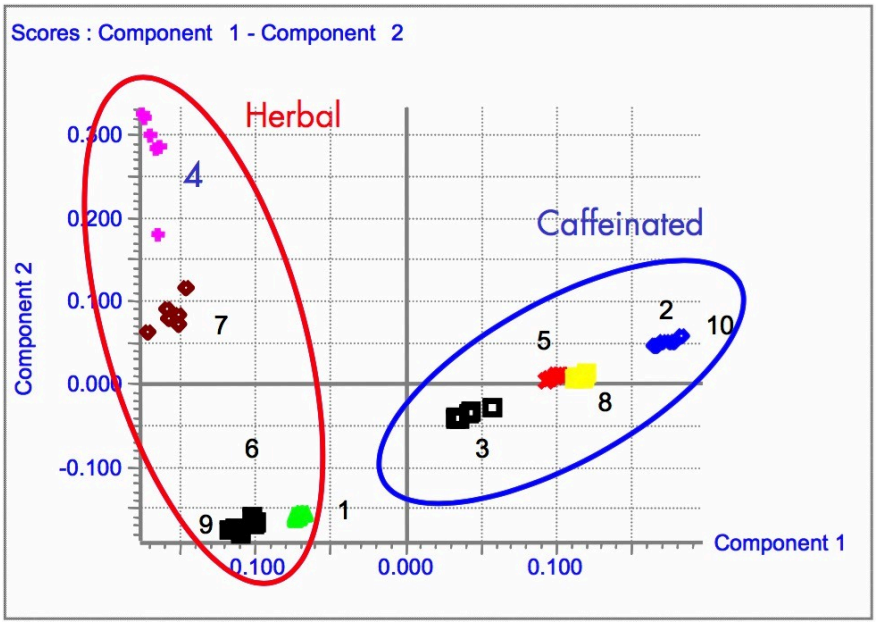

Figure 4. PCA scores plot showing the differentiation of the caffeinated and herbal infusion teas. Samples: (1) Tension Tamer, (2) Japanese Green, (3) Chai, (4) Cranberry, Raspberry, and Elderflower, (5) Traditional English, (6) Lemon and Ginger, (7) Black Current, Ginseng, and Vanilla, (8) Earl Grey, (9) Chamomile, and (10) Lotus Green.

The results in Figures 1 and 4 both show that it is possible to differentiate between the different tea varieties as well as caffeinated or herbal infusions based solely on UPLC-MS data using multivariate analysis.

The PCA scores plot in Figure 4 shows that Earl Grey and English Tea are statistically similar and likewise for Japanese Green and Lotus Green Teas, while Chai tea was dissimilar to both the black and green teas. The RT-*m/z* pairs which contribute most significantly to the observed clustering are shown in the PCA loadings plot.

In order to identify these unknown analytes, the superior mass accuracy (<3 ppm, RMS) of the LCT Premier XE allowed for confident database searching.

The results from an external database search indicates that the majority of the statistically significant hits are flavan-3-ols, otherwise known as catechins (Figure 5).[1,2,3]

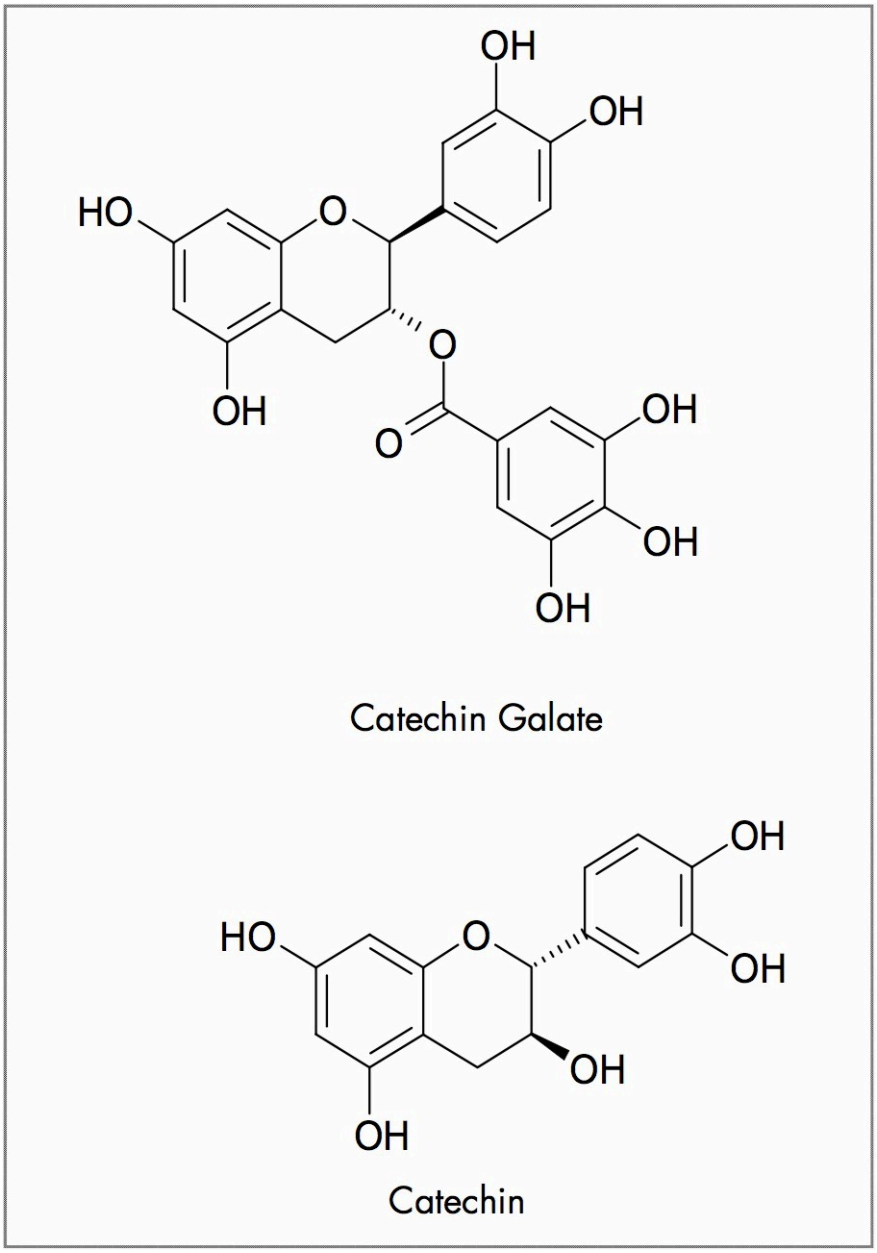

Figure 5. Examples of flavan-3-ols (commonly called catechins) that were identified in the tea dataset comparisons.

Identification of Unknowns

In Figure 6, the MarkerLynx trend plot (normalized ion intensity vs. sample) for catechin gallate illustrates a positive response for this analyte in the caffeinated teas only. The presence of catechin gallate in the caffeinated teas is in agreement with the published literature.[1,2,3] A significant number of the ions remained unidentified, even after the database search.

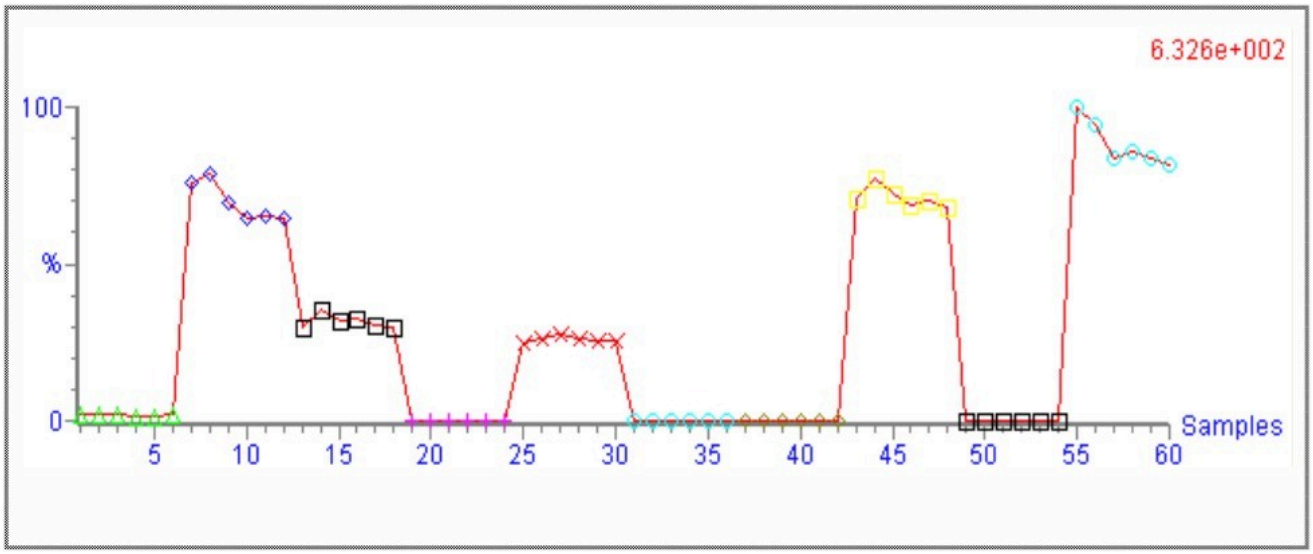

Figure 6. Trend plot for catechin gallate after performing exact mass searches using an external database.

Iced Tea Versus Hot Tea

To investigate any potential differences between hot and cold teas, samples were chilled after brewing and then analyzed by UPLC-MS. Inspection of Figure 7 demonstrates that the scores plot from the UPLC-MS data distinguishes between ice and hot brewed teas. These results further confirm that the 3-minute analysis time delivers more sample information in less time for this experimental study.

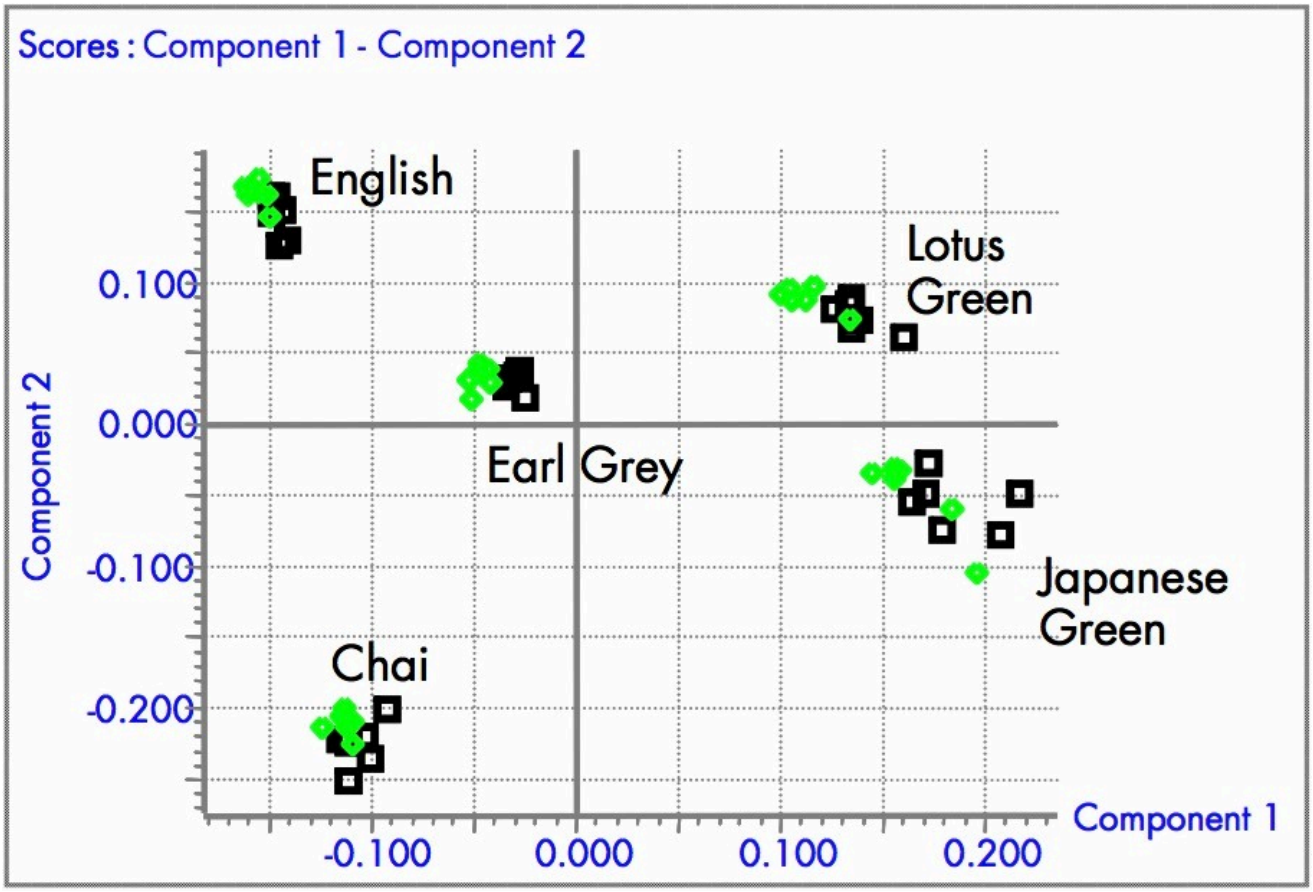

Figure 7. Scores plot illustrating group differences in freshly brewed (green diamonds) vs. iced (black squares) caffeinated teas.

Conclusion

This work demonstrates that by using the combination of the Waters ACQUITY UltraPerformance LC System and the LCT Premier XE Mass Spectrometer combined with the MarkerLynx Application Manager for MassLynx Software, it is possible to differentiate between tea varieties based solely on the UPLC-MS "fingerprint". Following statistical processing (PCA) within MarkerLynx, the exact masses provided by the LCT Premier XE formed the basis of searches against an external database. The search results indicated that the most statistically significant ions belonged to the catechin family of compounds. Speed of analysis was important in this study because the tea changed significantly as it was allowed to cool. Hence, the faster analysis time provided by the ACQUITY UPLC System allowed for rapid analysis of the tea samples before significant chemical side reactions could occur. There was no apparent loss of information from the fast 3-minute gradient when compared with longer 10- and 30-minute gradient separations.

References

1. Sakakibara Hiroyuki, Honda Yoshinori, Nakagawa Satoshi, Ashida Hitoshi, Kanazawa Kazuki. Simultaneous Determination of All Polyphenols in Vegetables, Fruits, and Teas. *J. Agric. Food Chem*. 2003, 51(3), 571–581.
2. Lin Yung-Sheng, Wu Sang-Shung, Lin Jen-Kun. Determination of Tea Polyphenols and Caffeine in Tea Flowers (Camellia sinensis) and Their Hydroxyl Radical Scavenging and Nitric Oxide Suppressing Effects. *J. Agric. Food Chem.* 2003, 51, 975–980.
3. Zeeb Daniel J, Nelson Bryant C, Albert Klaus, Dalluge Joseph J. Separation and Identification of Twelve Catechins in Tea Using Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry. *Anal. Chem*. 2000, 72(20), 5020–5026.

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