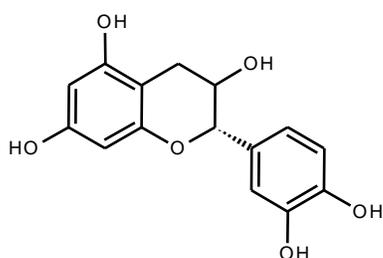


Application News

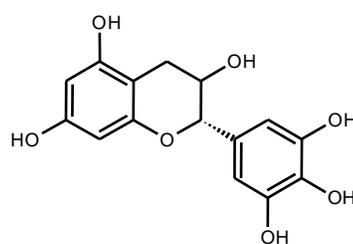
Liquid Chromatography Mass Spectrometry

Analysis of Polyphenols in Green Tea using the LCMS-IT-TOF

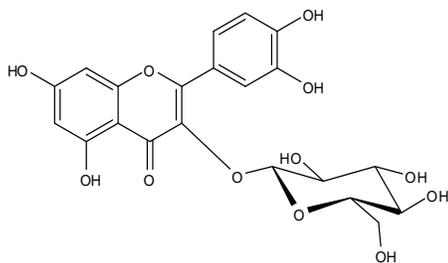
The polyphenols or catechins in green tea are known to possess many health benefits for individuals. These species possess antioxidative activity and are also thought to have chemopreventive effects against certain cancers. The antioxidative activity of catechins is theorized to help improve hypertension, reduce inflammation, and improve cognitive dysfunction in the elderly all of which are beneficial to one's health. The analysis of this class of compounds will continue to gain in importance as more individuals and companies (particularly those in the nutraceutical industry) discuss their potential health benefits.



catechin



gallocatechin



quercetin glycoside

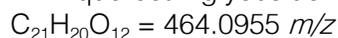


Figure 1: Structures for common polyphenols

Methods

Sample Preparation: One commercial green tea bag was boiled for 2 min in 250 mL H₂O. After cooling, the tea solution was divided equally and loaded on 6 preconditioned SPE columns (Supelco Supelclean LC-18, 3mL). The SPE columns were conditioned first with 3 rinses of 3 mL MeOH followed by 5 rinses of 3 mL of H₂O. After the samples were loaded, each column was rinsed 3 times with 3 mL's of H₂O. The polyphenols were then eluted with 833 μ L of extraction solvent, Acetone:H₂O:HAc (70:29.5:0.5), and the 6 fractions pooled to give a total volume of ~ 5 mL. The solution was centrifuged for 5 min at 7000 rcf and a 1:10 dilution used as the final working solution.

The green tea extract was then analyzed on the LCMS-IT-TOF using the conditions found in Table I. Formulas for polyphenols were predicted using Shimadzu's Composition Formula Predictor.

Table I: Analytical Conditions for LCMS-IT-TOF

Column	Shim-pack VP-ODS (2.0 mm x 150 mm)
Mobile phase A	0.5% Acetic Acid in H ₂ O
Mobile phase B	0.5% Acetic Acid in ACN
LC Time program (linear gradient)	5 - 12% B (0-5 min); 12 - 40% B (5-30 min); 40 - 70% B (30 - 35 min); 70% B (35 - 36 min); 70 - 5% B (36 - 38 min); 5% B (38 - 42 min)
Flow rate	0.2 mL/min
Injection volume	2.0 μ L
Probe voltage	4.50 kV
CDL temperature	200 °C
Block heater temperature	200 °C
Nebulizing gas flow	1.5 L/min
Ion accumulation time	30 msec
MS (-ESI)	100 - 1500 <i>m/z</i> ,
MS ²	50 - 1100 <i>m/z</i> ; CID - Energy 35%; Collision gas 50%; time 30 msec
MS ³	50 - 800 <i>m/z</i> ; CID - Energy 75%; Collision gas 75%; time 30 msec

Results

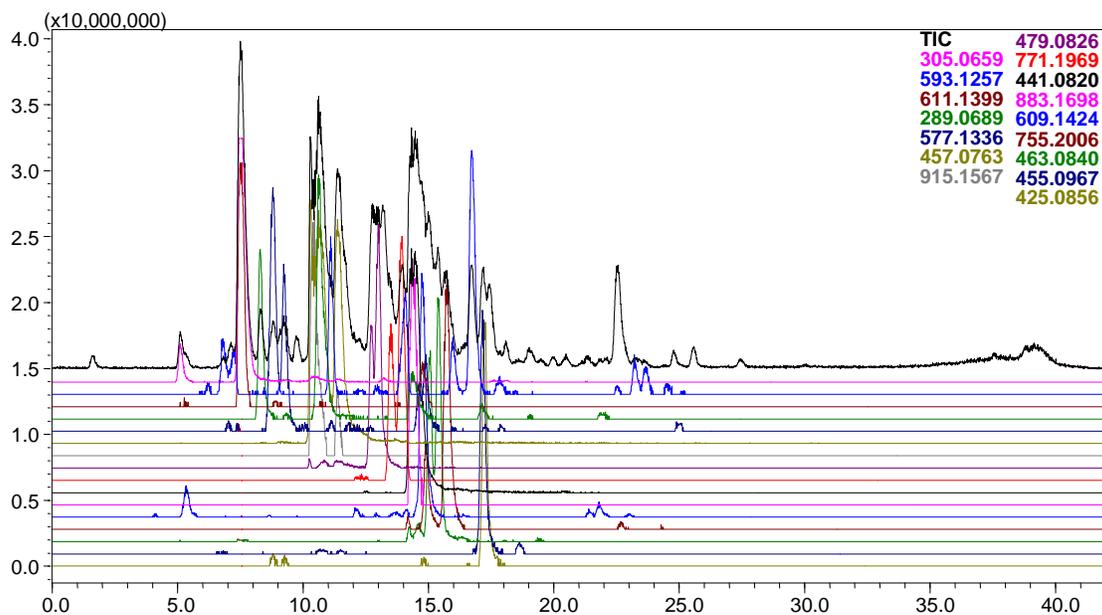


Figure 2: (-) ESI MS Chromatograms for the separation of the polyphenols in green tea

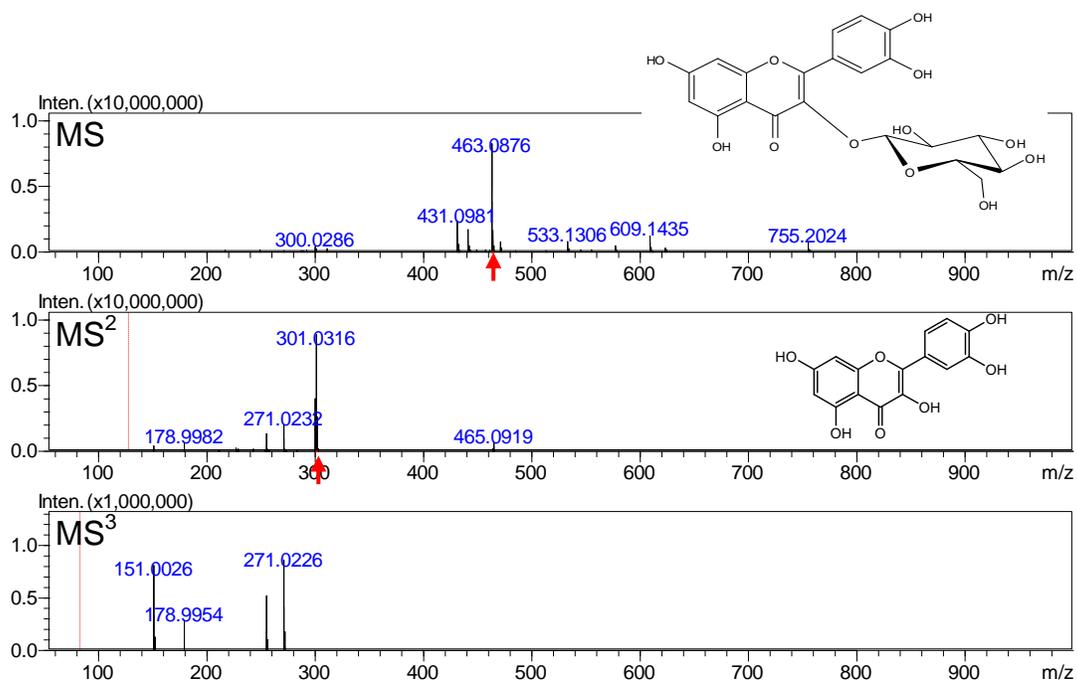


Figure 3: MS spectra showing dissociation of a quercetin glycoside at 463 m/z. Red arrows indicate which ions were chosen for subsequent MS/MS analysis. Structures for the parent ions are provided.

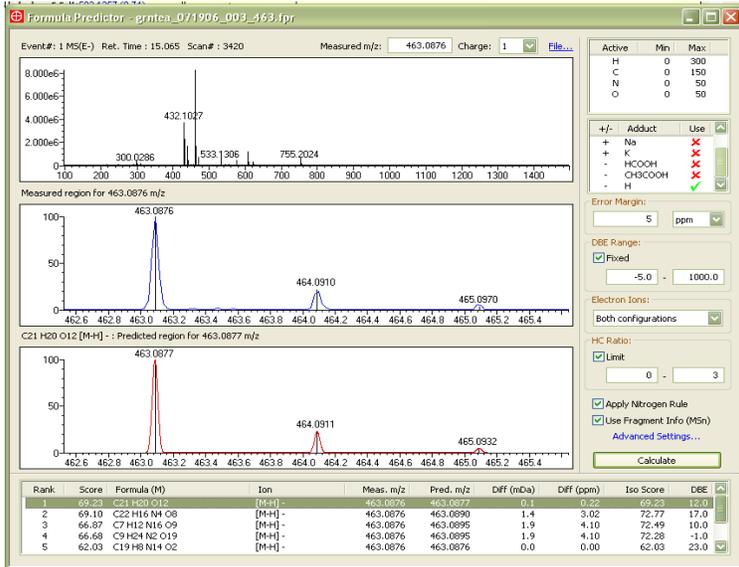


Figure 4: Composition Formula Predictor results for the 463 m/z ion. The #1 ranked formula is a known catechin found in green tea.

Table II: Mass Accuracy for catechins observed in the analysis of green tea using the LCMS-IT-TOF

Name	Formula [M]	m/z Calculated (monoisotopic)	m/z Observed	Mass Accuracy (ppm)
galocatechin	C ₁₅ H ₁₄ O ₇	305.0661	305.0658	0.98
galocatechin	C ₃₀ H ₂₈ O ₁₄	611.1401	611.1390	1.80
catechin	C ₁₅ H ₁₄ O ₆	289.0712	289.0696	5.53
myricetin glycoside	C ₂₁ H ₂₀ O ₁₃	479.0826	479.0820	1.25
quercetin glycoside	C ₂₁ H ₂₀ O ₁₂	463.0877	463.0876	0.22
quercetin glycoside	C ₂₇ H ₃₀ O ₁₅	593.1506	593.1486	3.37
quercetin glycoside	C ₂₇ H ₃₀ O ₁₆	609.1456	609.1452	0.66
quercetin glycoside	C ₃₃ H ₄₀ O ₂₀	755.2035	755.2029	0.79
quercetin glycoside	C ₃₃ H ₄₀ O ₂₁	771.1984	771.1949	4.54

Conclusions

The LCMS-IT-TOF provides powerful data due to its ability to collect MSⁿ data and provide good mass accuracy. Most of the catechins observed in the Green Tea extract were detected with a mass accuracy of 5 ppm or less. Combining the MSⁿ fragmentation information with the mass accuracy of the parent compound allows an individual to predict a formula for their unknowns with a greater degree of certainty using Shimadzu's Composition Formula Predictor.