

# Metabolomic Analysis of Green and Black Tea Extracts Using an LTQ Orbitrap XL Hybrid Linear Ion Trap Mass Spectrometer

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## Overview

**Purpose:** To show a complete analytical metabolomic workflow including (1) data acquisition using a high resolution accurate mass instrument that is equipped with a Higher Energy Collisional Dissociation (HCD) cell and coupled to a high pressure LC (Figure 1), (2) metabolite differential abundance analysis, and (3) structural elucidation of relevant metabolites using accurate mass and HCD fragmentation information to highlight the component differences between green and black tea.

**Methods:** Green and black tea extracts were analyzed using an LTQ Orbitrap XL™ with an HCD cell. Chromatography was performed using an Accela High Speed LC equipped with a 2.1 mm ID Hypersil GOLD™ column packed with 1.9 μm particles. Data Dependent™ analysis was performed using an LTQ Orbitrap XL with full scan data acquired at a resolving power of 30,000 and MS<sup>n</sup> data acquired at a resolving power of 7500 following HCD fragmentation.

**Results:** The study included a comparative analysis of green and black tea using differential analysis software to identify compositional variations between the two tea samples. Using a UHPLC coupled with a small particle column afforded a fast analysis time while maintaining very high chromatographic resolution. The high mass accuracy data (better than 3 ppm with external calibration) was used to determine elemental composition and for tentative identification of compounds via database searching. HCD fragmentation facilitated structural identification and confirmation. This was demonstrated with the example of epigallocatechin gallate (EGCG).

## Introduction

Metabolomics, the comprehensive and quantitative analysis of wide arrays of metabolites in biological samples, marks promising new research territory. The numerous analytes in these samples have diverse chemistries and polarities. In addition, metabolites occur at a range of concentrations within a particular sample. Consequently, comprehensive metabolomics investigations create many analytical challenges that can be addressed using LC-MS/MS.

Tea contains a wide range of components including vitamins, amino acids, and polyphenols, many of which are structurally similar and may differ only in the type and location of a side chain. The use of high resolution chromatography is essential for the separation of such a complex mixture. Furthermore, acquisition of accurate mass data in both full scan and MS<sup>n</sup> modes enables complete structural characterization.

Here, we highlight an untargeted metabolomic workflow from data acquisition through metabolite ID. The study included differential and structural characterization of polyphenolic catechin (flavan-3-ol) derivatives and theaflavin components of green tea and black tea.

## Methods

### Samples

Green tea and black tea aqueous extracts were examined without any pre-treatment. Each sample was analyzed in quadruplicate.

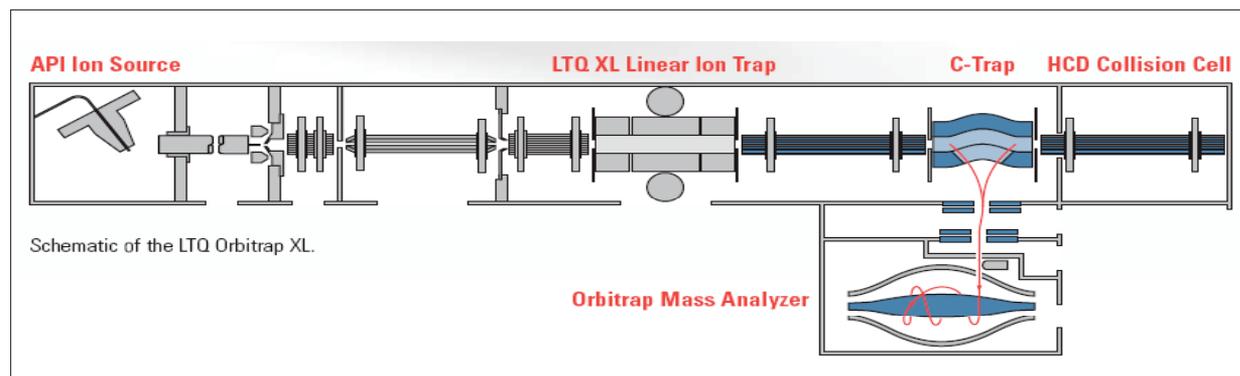


Figure 1: Schematic of the LTQ Orbitrap XL mass spectrometer with an HCD collision cell. The LTQ Orbitrap XL features an HCD collision cell to provide additional flexibility for low level components in complex mixtures. Ions can be selected in the linear ion trap and fragmented either in the ion trap (CID) or in the HCD collision cell. For HCD, ions are passed through the C-trap into the gas-filled collision cell, providing high sensitivity and high signal-to-noise fragmentation.

## Key Words

- Accela™ High Speed UHPLC
- SIEVE™ Software
- Higher Energy Collisional Dissociation (HCD)
- Mass Frontier™ Software
- Natural Product Analysis

## Chromatography Conditions

Chromatographic separation was performed using the Accela UHPLC system (Thermo Fisher Scientific, San Jose, CA). The LC conditions were as follows:

Column: Hypersil GOLD, 100 × 2.1 mm, 1.9 μm particle size (Thermo Fisher Scientific, Bellefonte, PA)

Mobile phase: (A) water with 0.1% formic acid;  
(B) acetonitrile with 0.1% formic acid

Flow rate: 500 μL/min

Injection volume: 10 μL

Gradient: Linear gradient of 100%–1% A over 20 minutes

## Mass Spectrometry Conditions

MS analysis was carried out using an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The MS conditions were as follows:

Positive electrospray ion source voltage: 5.0 kV

All methods: Full scan MS in the Orbitrap with a mass resolution of 30,000. Data Dependent MS/MS in the Orbitrap on the top three most intense ions from the full scan at a mass resolution of 7500.

## Results

Considerable interest has developed in the potential health benefits of teas, particularly in the antioxidant and other properties of some of the polyphenolic catechins and theaflavins (Figure 2). The analysis described here focused on detection, relative quantitation, and identification of these low molecular weight components in green and black tea samples.

The HPLC separation of tea samples shows excellent peak separation and low noise, with most components eluting in less than 10 min. High resolution full scan spectra were acquired at a mass accuracy of better than 3 ppm.

After data acquisition, SIEVE software was used to determine statistically significant differences in the metabolite profiles of green and black tea samples (Figure 3). By comparing peak intensities between the two chromatographically aligned samples, metabolites present at different levels in the two teas were identified.

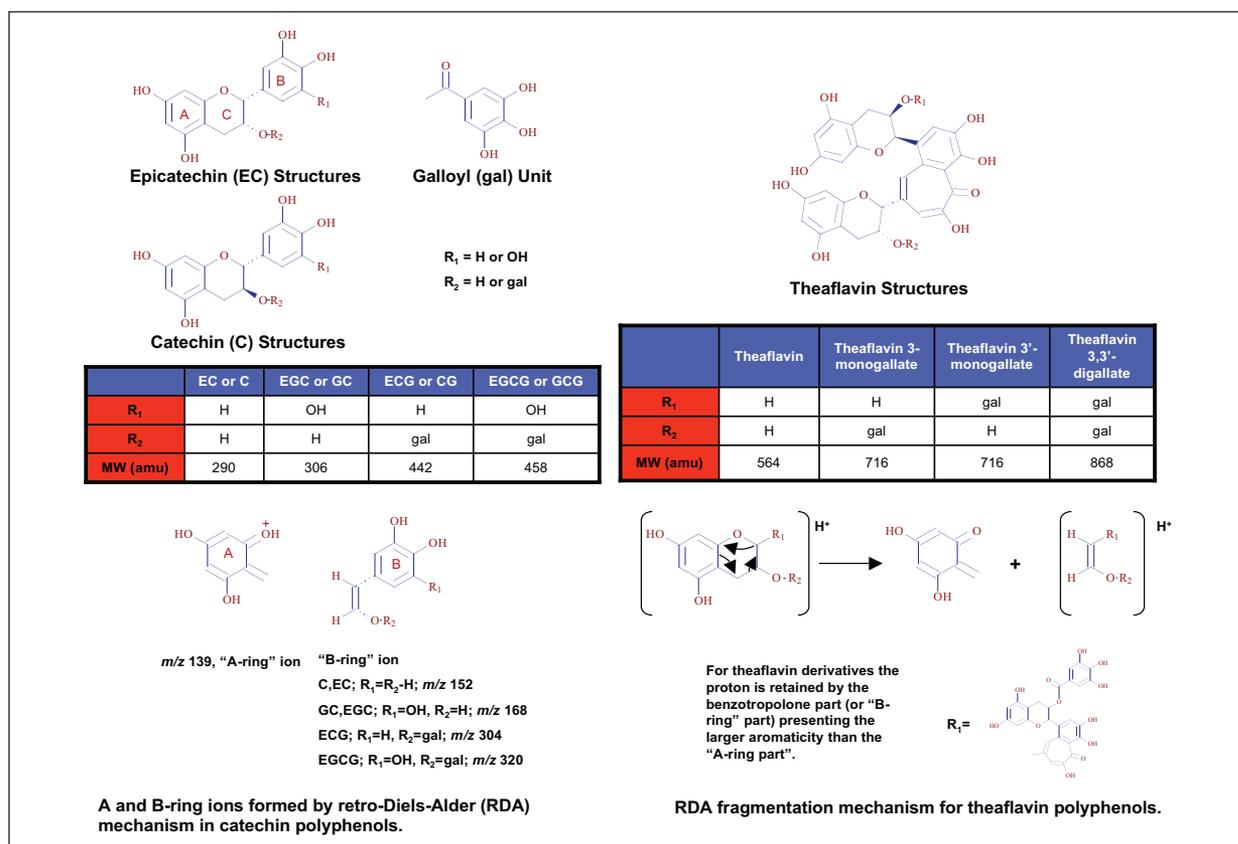


Figure 2: Three types of tea are produced from *Camellia sinensis* leaves: green tea (nonfermented), oolong tea (semi-fermented), and black tea (fermented). Catechins are polyphenolic antioxidant plant metabolites of the class flavanoids called flavan-3-ols and are highly present in tea plants. Fermentation induces enzymatic oxidation of flavan-3-ols and leads to the formation of two major pigments in black tea, theaflavins (TFs) and thearubigins (TRs). Catechins are expected to be more abundant in green tea and theaflavins more abundant in black tea. The proposed fragmentation pathway for these compounds proceeds via a Retro-Diels-Alder rearrangement as outlined here.

After differentially abundant metabolites of interest were detected, the accurate mass and the MS<sup>n</sup> data were used for structural identification. The elemental formula, as determined by the accurate mass data, and the accurate mass value itself were used for metabolite database searching. The EGCG metabolite was tentatively assigned using this combination of tools.

Further metabolite characterization was accomplished using MS<sup>n</sup> spectra and Mass Frontier software. Mass Frontier allowed confident metabolite identification using its comprehensive spectral library and predictive fragmentation algorithms to facilitate structural elucidation (Figure 4). The compounds in Figure 3 were identified using this workflow.

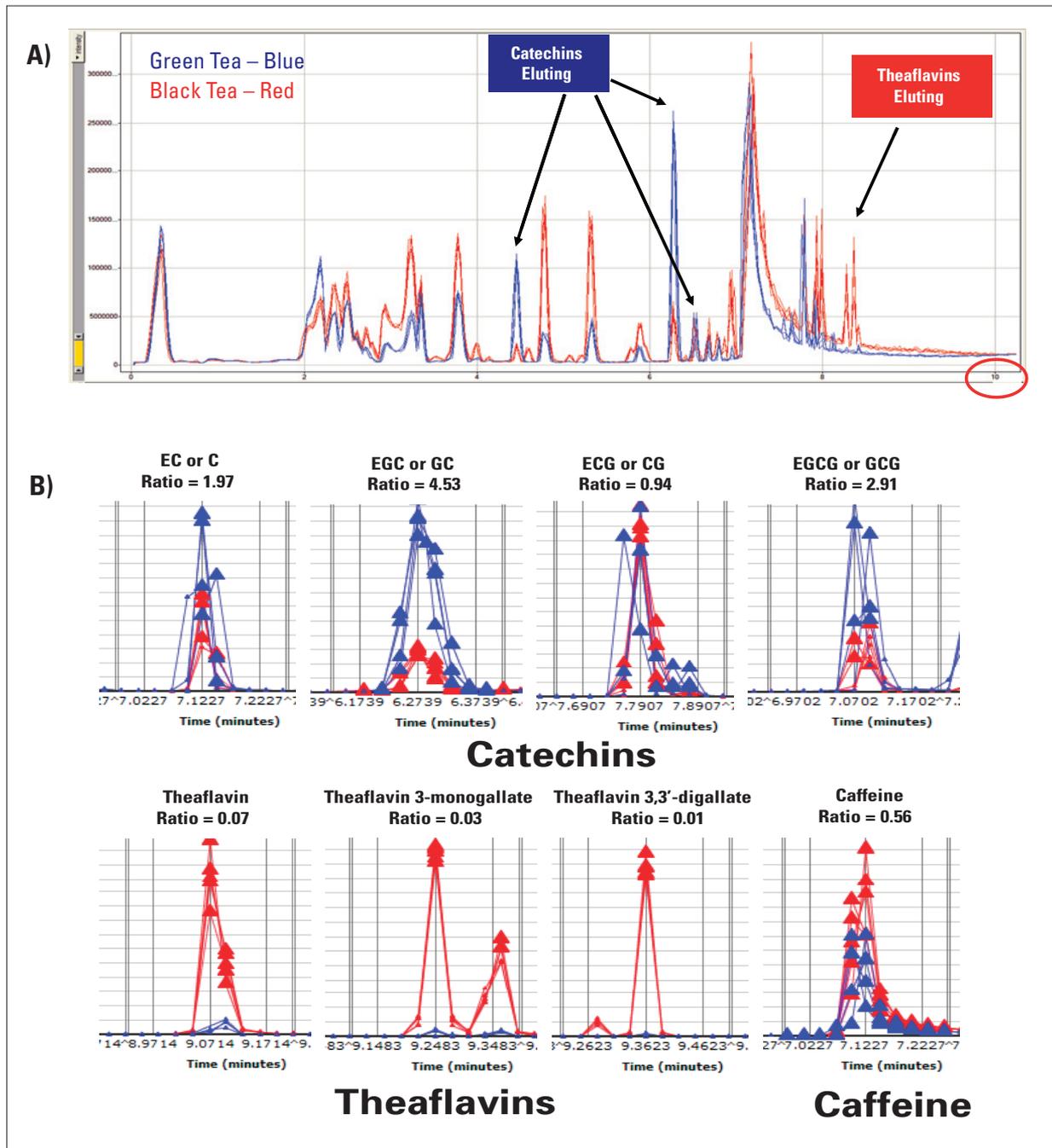


Figure 3: Differential metabolite abundance analysis with SIEVE software. A) Chromatographic alignment of the various LC sample traces is the first step in the SIEVE process. Differences between the green tea samples (Blue) and the black tea samples (Red) can be identified. The Accela UHPLC provided highly resolved chromatographic peaks and high signal-to-noise ratios. B) After alignment, the corresponding peak intensities are compared for green tea (Blue) and black tea (Red). The relative abundances of several compounds of interest are shown with their abundance ratios. These metabolites were identified using a combination of accurate mass database searching and MS<sup>n</sup> spectra interpretation via Mass Frontier software.

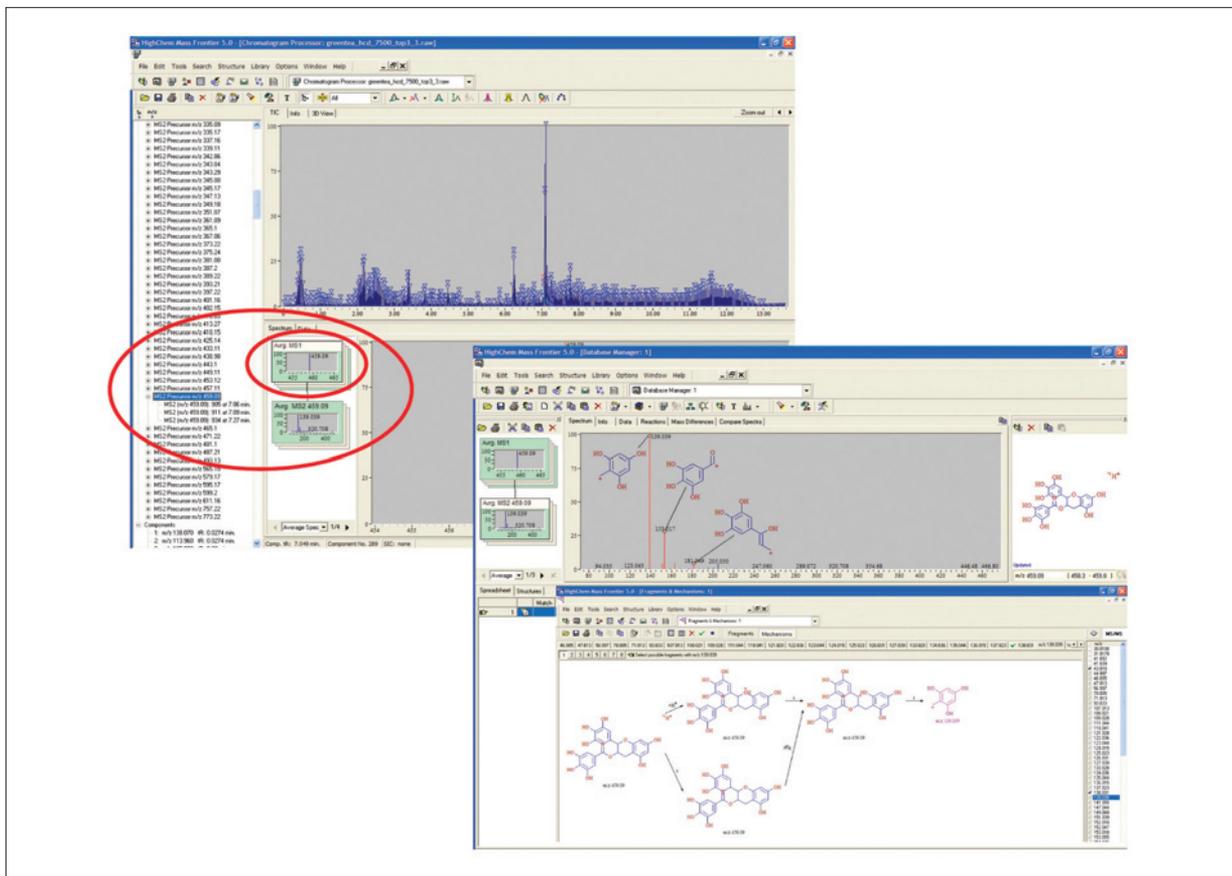


Figure 4: A metabolite of interest from Figure 3 was chosen for further characterization. This ion was present at levels ~3 times that of black tea and was identified as the potent antioxidant, EGCG. Accurate mass was used to identify the metabolite via database searching, and Mass Frontier software was used to confirm the EGCG identification by using the fragmentation spectra of the parent ion.

## Conclusions

The analytical metabolomic workflow described here encompasses data acquisition, discovery of differentially abundant metabolites, and metabolite identification. The LTQ Orbitrap XL coupled to an Accela U-HPLC system afforded fast analysis times while maintaining high chromatographic resolution. Accurate mass measurements increased the confidence in elemental composition assignments and ultimately metabolite identification. SIEVE

differential analysis software enabled large-scale evaluation of multiple complex LC-MS data and comparison of metabolite profiles between green and black tea samples. The spectral database and fragmentation algorithms of Mass Frontier software facilitated structural assignments of metabolites of interest utilizing MS<sup>n</sup> fragmentation spectra.

## References

Menet, M.C., Sang, S. Yang, C.S., Ho, C.T., and Rosen, R.T., *Analysis of Theaflavins and Thearubigins from Black Tea Extract by MALDI-TOF Mass Spectrometry*. *J. Agric. Food Chem.* 2004, 52, 2455-2461.

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