Delivering the Right Results

Characterization of Nutrients and Actives in Herbal Supplements and Nutriceuticals Using UHPLC-Multi-Reflecting Time-of-Flight Mass Spectrometry—Comparison of Suppliers and Dosage Forms of Green Tea, Ginger, and Acai Berry Extracts

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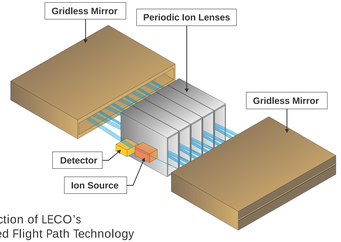
INTRODUCTION

Abstract–The qualitative and quantitative analysis of the content of nutritional supplements and alternative medicinal therapies is becoming more important, and regulation by the FDA is imminent. High resolution mass spectrometry provides a rapid, selective, and useful mechanism for QC of extracts and finished dosage forms, producing a minable, comprehensive data set. In this application, extracts and various supplement dosage forms are introduced via direct infusion or UHPLC separation and analyzed by mass spectrometry (MS). Ultra-high resolution MS was performed using a multi-reflecting time-of-flight MS (MR-TOFMS). The analyses provided chemical fingerprints which are qualitatively and quantitatively distinct. The utility of these resolving powers and acquisition speeds for facile determination of elemental composition and resultant unknown identification is demonstrated. The utility of in-source CID (isCID) to provide supplemental structural information is also presented.

Background–"Nutriceuticals" is a word coined from nutritive and pharmaceuticals and captures herbal and similar botanical extracts used for health and pharmaceutical purposes. It is a growing industry with its foundations in early pharmaceuticals and in the natural products world. Some individuals use these compounds as a primary source of therapeutics. The US Food and Drug Administration has suggested that in order to ensure the efficacy of these therapeutics and to standardize the activity, quality control measures may be enacted. One of the measures which shows promise is the use of mass spectrometry to monitor the highly complex mixtures which are often found in the dosage forms, which vary from teas to capsules to liquids. Often it is not one but the combination of several components, some of which may be "additives" or supplements, which contribute to the therapeutic activity. The ability to track active ingredients and identify adulterants, and thereby provide a measure of consistency and control in the composition and activity, is important. Electrospray ionization interfaced to high performance TOF mass spectrometry is used to characterize and compare various dosage forms of several common nutriceuticals. The mass spectrometer used is an innovative instrument which employs Folded Flight Path™ (FFP™) technology to provide high resolution, accurate mass, and accurate isotope abundance.

Green Tea Ginger Acai Berries



Depiction of LECO's Folded Flight Path Technology

EXPERIMENTAL

Materials Examined and Their Contents

Green Tea Forms	Organic Leaf Green Tea; Green Leaf Tea; Green Tea Extract; Decaffeinated Green Tea; Supplement containing Green Tea and Acai Berry Extract
Acai Berry Forms	Berry Sample 1; Berry Sample 2
Ginger	Chopped Ginger

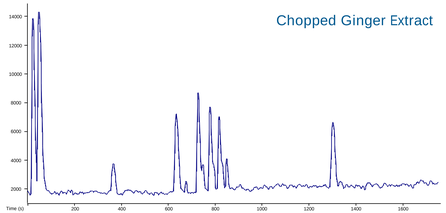
Each of these was extracted into HPLC grade water by shaking for 30 minutes at 65°C then diluted 10x into 0.2% HFBA prior to HPLC analysis.

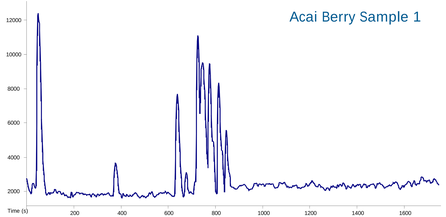
LC	Agilent 1200/Agilent 1290
Column	1.9 µm Hypersil Gold 50 mm or 30 mm x 1.0 mm ID
Mobile Phases	0.1% formic acid in water (A), acetonitrile (B)
Slow Gradient	5 min 0% B; to 80% AcN in 40 min at 0.1 mL/min
Fast Gradient	0.5 min 0% B; to 80% AcN in 3 min 0.25 mL/min
Column Temperature	40°C
Injection Volume	2.5 µL
Mass Spectrometer	LECO CITIUS™ LC-HRT
Ion Source	LECO ESI—Positive polarity
Acquisition Mode	High Resolution (ca. 50,000 resolving power)
Fragmentation Mode	Pulsed isCID at 20 V differential
Spectral Acquisition	2 or 40 spectra/second (split equally between parent/fragment)
m/z Range	50 to 1750
m/z Calibration	External with Agilent tuning mix by co-infusion w/effluent

TARGETED ANALYTES FOR NUTRICEUTICALS

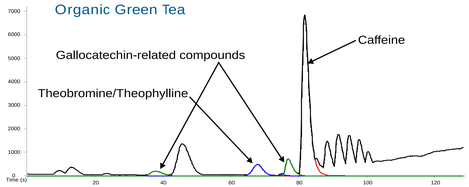
Name	Formula	Monoisotopic Masses $[M+H]^+$
Quinic Acid	$C_7H_{12}O_6$	191.05501
Coumaryl-quinic acid	$C_{16}H_{18}O_8$	338.09962
Gallic Acid	$C_7H_6O_5$	169.01315
Theogallin	$C_{13}H_{16}O_{10}$	343.06597
Trigalloyl-glucose	$C_{27}H_{22}O_{18}$	507.12857
Digalloyl-glucose	$C_{20}H_{20}O_{14}$	635.08789
Corilagin	$C_{27}H_{22}O_{18}$	483.07693
Epicatechin	$C_{22}H_{20}O_{10}$	633.07224
Epigallocatechin	$C_{22}H_{20}O_{10}$	441.08162
Epigallocatechin gallate	$C_{29}H_{20}O_{11}$	457.07654
Epicatechin	$C_{15}H_{14}O_6$	289.07066
Epigallocatechin	$C_{15}H_{14}O_7$	305.06558
Gallocatechin	$C_{15}H_{14}O_7$	305.06558
Kaempferol	$C_{15}H_{10}O_6$	287.05501
Quecetin	$C_{15}H_{10}O_7$	321.06049
Myrecitin	$C_{15}H_{10}O_8$	319.04484
Caffeine	$C_8H_{10}O_2N_4$	195.08765
Theobromine	$C_7H_8O_2N_4$	181.072
Theophylline	$C_7H_8O_2N_4$	181.072
Theamine	$C_7H_{14}N_2O_2$	175.10772

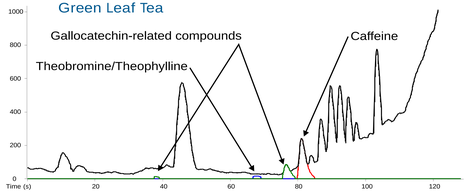
(These are select analytes likely to be in Green Tea, Ginger or Acai)

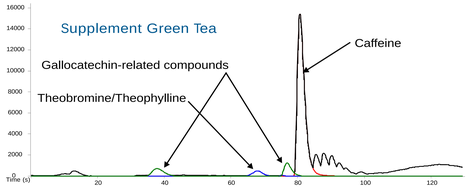


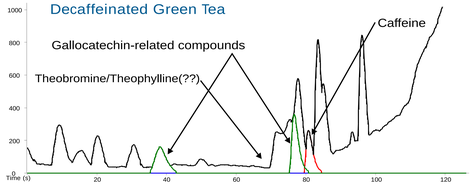


REPRESENTATIVE RESULTS









REPRESENTATIVE RESULTS

Of the compounds in the table, those shown below were identified in the samples analyzed. It is suspected that the gentle extraction conditions, designed to mimic "steeping" tea, were not sufficient to extract some of the more hydrophobic or deeply residing compounds. In all instances, when detected, the mass error is less than 2 ppm, even under low signal conditions.

Name	Formula	Theoretical	Green Tea	Decaf	Organic	Supplement Green Tea
Caffeine	$C_8H_{10}O_2N_4$	195.08765	195.08696	195.08752	195.08713	195.08722
Theobromine	$C_7H_8O_2N_4$	181.07200	181.07168	nd	181.07211	181.07207
Theophylline	$C_7H_8O_2N_4$	181.07200	181.07168	nd	181.07211	181.07207
Theamine	$C_7H_{14}N_2O_2$	175.10772	nd	nd	175.10766	175.10767
Epigallocatechin_like	$C_{15}H_{14}O_7$	307.08123	307.08147	307.08097	307.08103	307.08131
Epigallocatechin_like_2	$C_{15}H_{14}O_7$	307.08123	307.08046	307.08034	307.08078	307.08091
Ephedrine-like	$C_9H_{14}NO_2$	152.07061	152.07031	152.07032	nd	152.07046
UNKNOWN	$C_{15}H_{24}N_2O_6$	337.16054	337.15924	337.1607	337.16044	nd
UNKNOWN	$C_7H_5Cl_2O_2$	144.98176	144.8141	nd	nd	nd

To further investigate the identity of the unknowns, relative isotope abundance was compared to the theoretical values. The table below shows the relative isotope abundances for the unknowns from the most concentrated sample. Three analytes had insufficient signal (NSS) to measure the isotope ratio. The ephedrine-like compound provided an outstanding isotopic match, and that for the fifth compound clearly confirmed the presence of a Cl in that compound.

Analyte	Formula	Theor. m/z	1st isotope	2nd isotope	3rd Isotope
Epigallocatechin_like	$C_{15}H_{14}O_7$	307.08123	NSS	NSS	NSS
Epigallocatechin_like_2	$C_{15}H_{14}O_7$	307.08123	NSS	NSS	NSS
Ephedrine-like	$C_9H_{14}N_1O_2$	152.07061	100.5	103.9	NSS
UNKNOWN	$C_{15}H_{24}N_2O_6$	337.16054	NSS	NSS	NSS
UNKNOWN	$C_7H_5Cl_1O_2$	144.98176	118.4	167.9*	88.2

*Signal present but weak.

SUMMARY AND CONCLUSIONS

High performance mass spectrometry has been used to differentiate various forms of common nutriceuticals with respect to their "nutrient" content. Differences in analyte concentration were measured by relative abundance of select analytes in the different forms. Identification was achieved using accurate mass determination with measured mass accuracies of typically less than 1 ppm. In addition, relative isotope abundance measurements provided a mechanism by which to support the formula of putative analytes. The analysis also detected five analytes with well-defined m/z values but remain unknowns.

The analysis of Green Tea samples was achieved with both HPLC (not shown here) and UHPLC. The analysis time in UHPLC was less than 2 minutes at 40 spectra/second and provided the differentiating results shown above. High performance mass spectrometry cannot provide direct evaluation of the nutritional content of natural extracts, but it is capable of providing a high information content fingerprint. This fingerprint may ultimately provide the needed information on the key "active ingredients" in certain nutriceuticals. Theamine, theophylline, and caffeine were elevated relative to the other Green Teas in the organic green tea and in the Green Tea Supplement samples. High performance MS was implemented using FFP™ technology and provided acquisition speed, mass accuracy, and isotopic integrity with *No Compromises*.