

Characterization of Nutrients and Actives in Herbal Supplements using UHPLC-Multi-reflecting Time-of-flight MS

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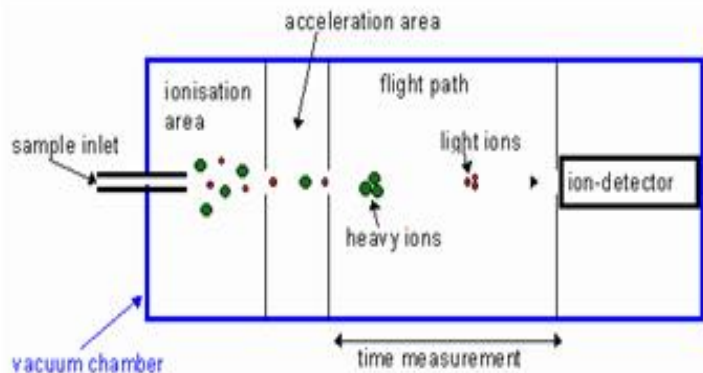
Overview

- Introduction
 - TOF-Principles
 - Multi-Reflecting TOF
 - LC-HRT Instrumentation
- Sample Preparation and LC-HRT Method Parameters
- Discussion of the Results
- Summary and Conclusions

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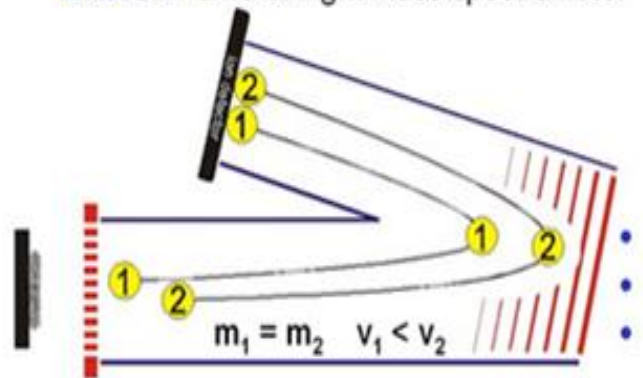
TOF Principles



Linear time-of-flight mass spectrometer

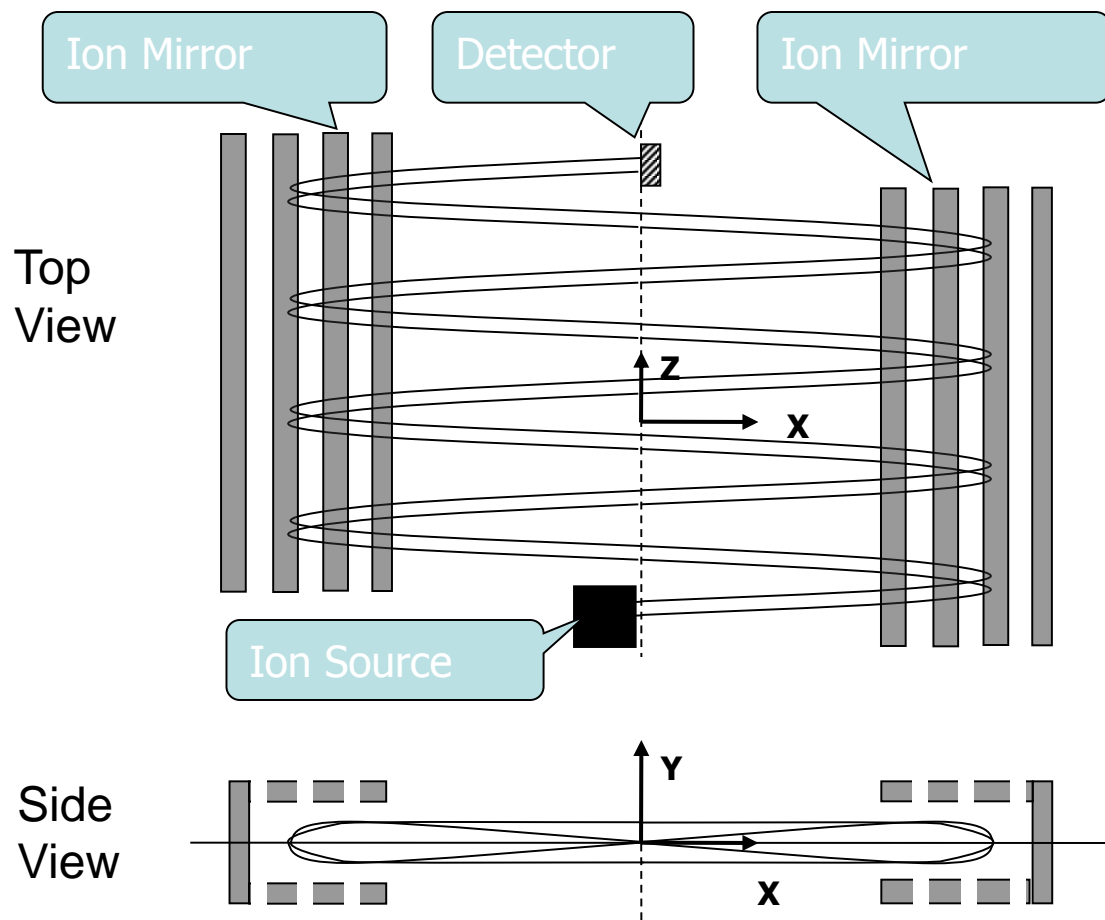


Reflector time-of-flight mass spectrometer



$$Flight_time = \frac{drift_length}{velocity} = drift_length \times \sqrt{\frac{mass}{2 \times energy}}$$

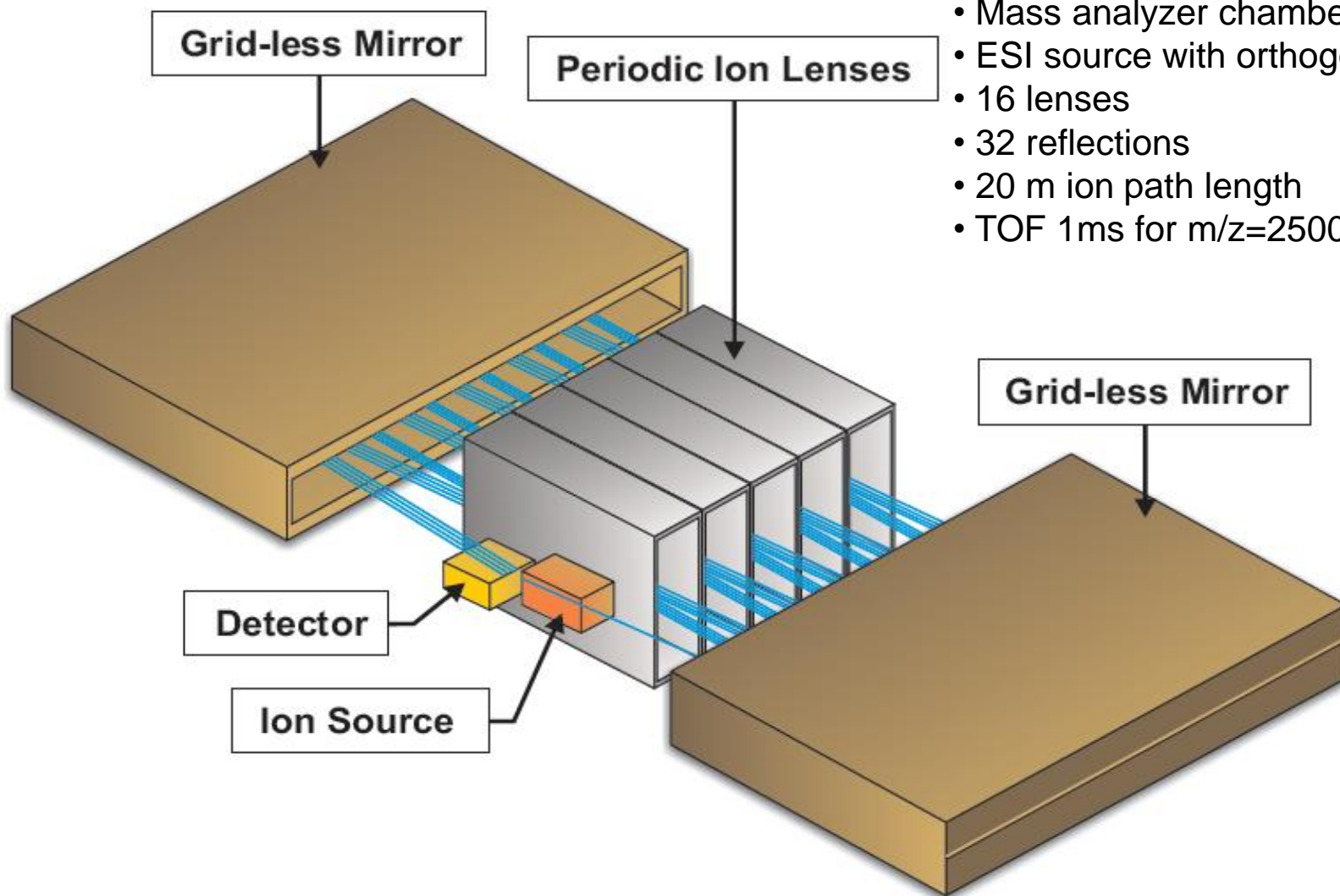
Multi-Reflecting TOF



Folded **F**light **P**ath
Technology

Nazarenko et.al.
SU 1725289 (1989)

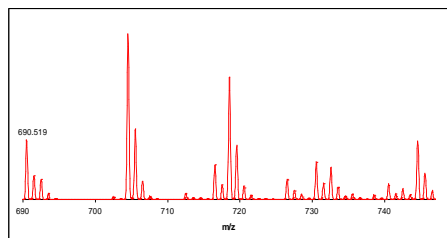
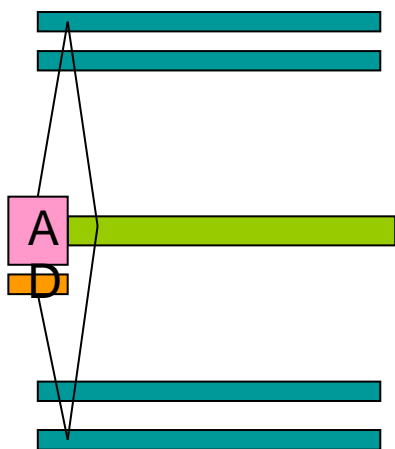
MRT Flight Tube Design



- Mass analyzer chamber 75x15x10cm
- ESI source with orthogonal injection
- 16 lenses
- 32 reflections
- 20 m ion path length
- TOF 1ms for $m/z=2500$

Modes of Analyzer Operation

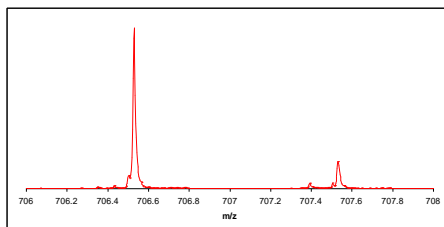
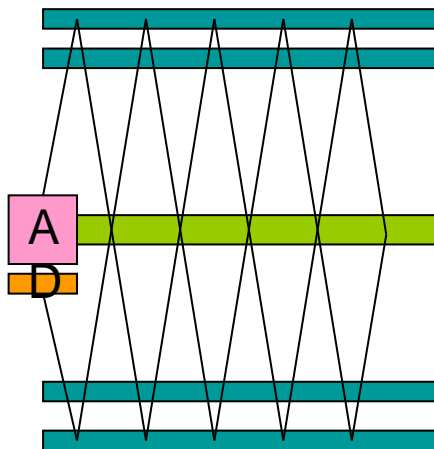
Nominal:
Sample Screening



R=2,500

Mass Accuracy: nominal
Full Mass Range

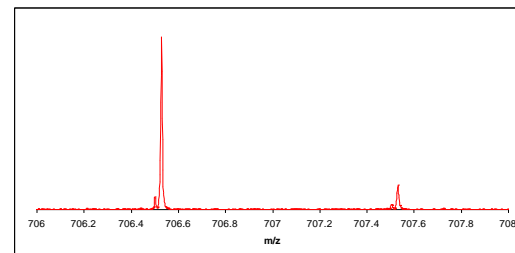
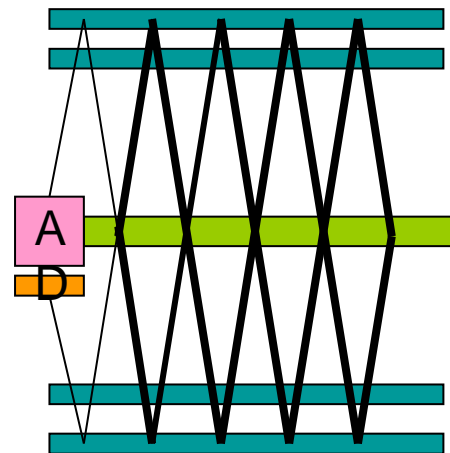
High Resolution:
Identification



R=50,000

Mass Accuracy: <1ppm
Full Mass Range

Ultra-High Resolution
Confirmation



R=100,000

Mass Accuracy: <1ppm
4:1 mass Range

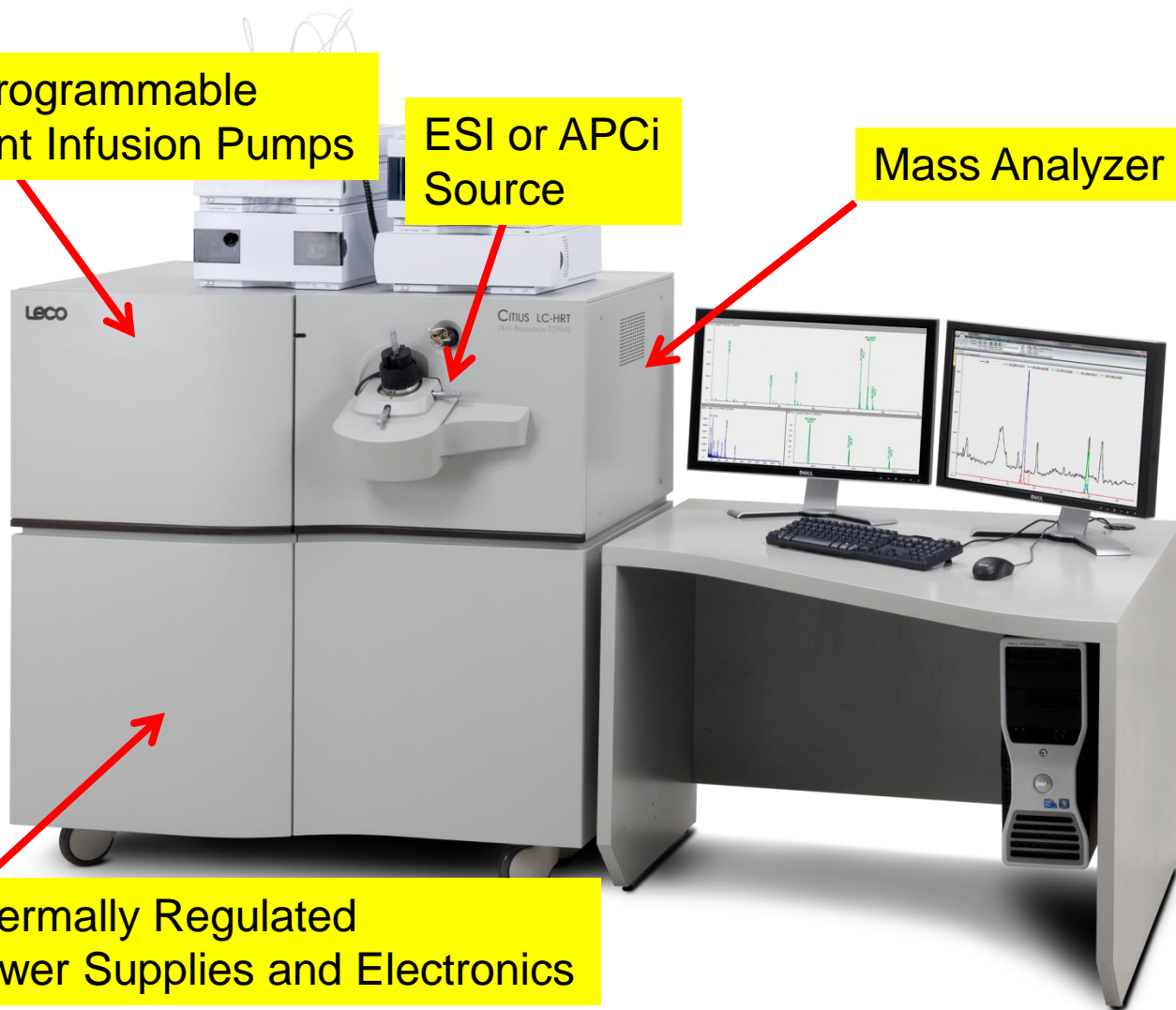
Up to **200 spectra/sec** in any resolution mode

LECO Citius® LC-HRT

Time Programmable
Calibrant Infusion Pumps

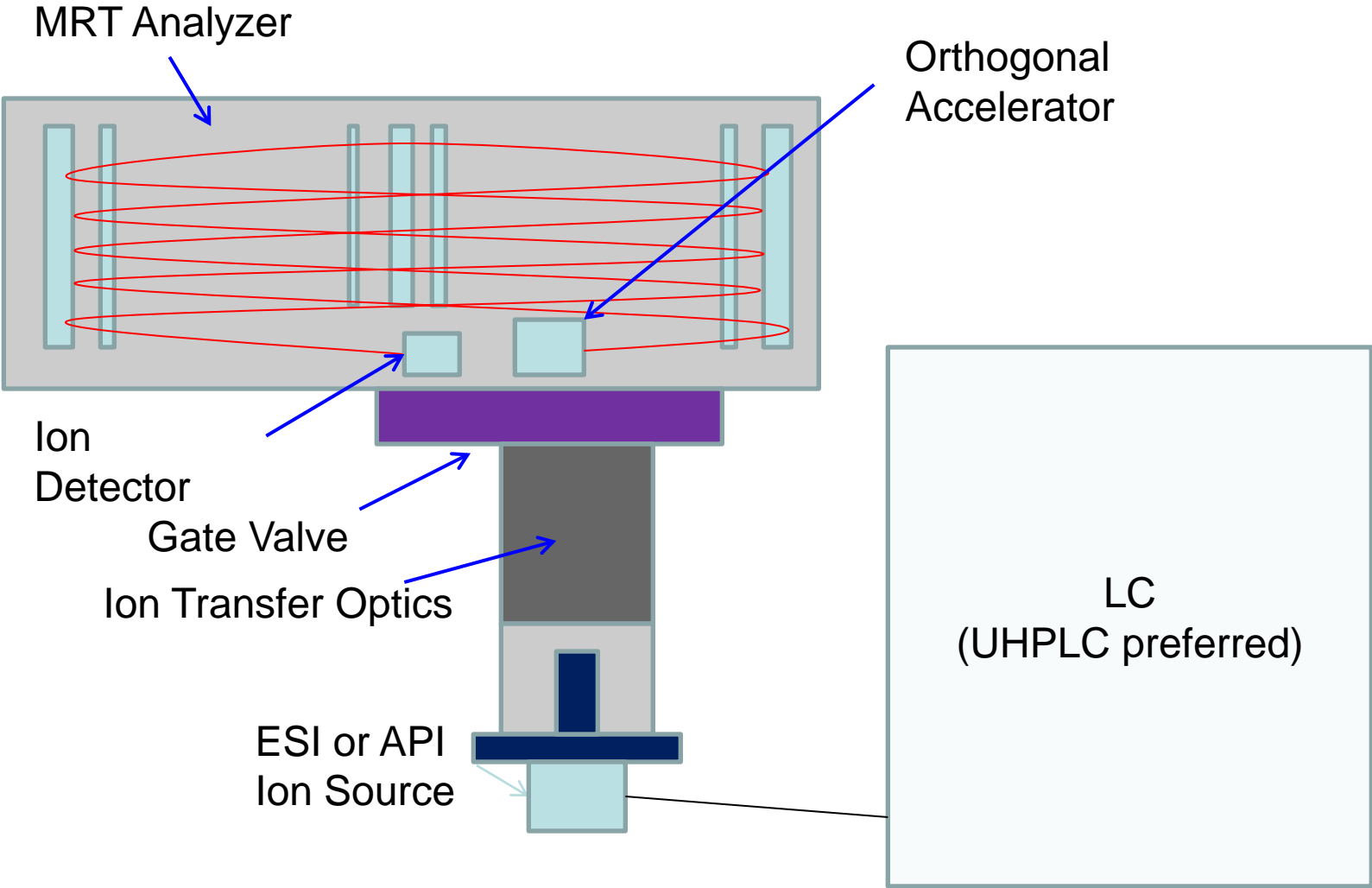
ESI or APCi
Source

Mass Analyzer

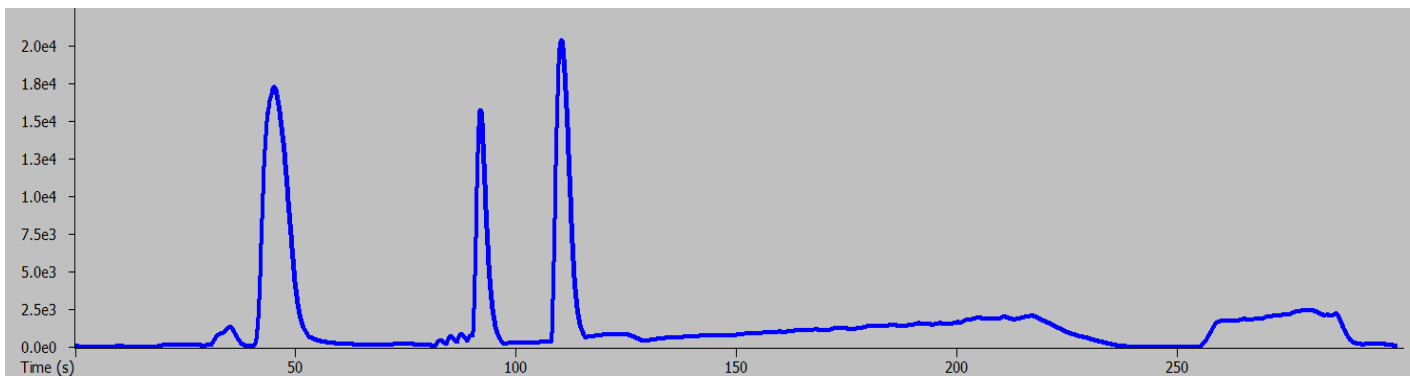


Thermally Regulated
Power Supplies and Electronics

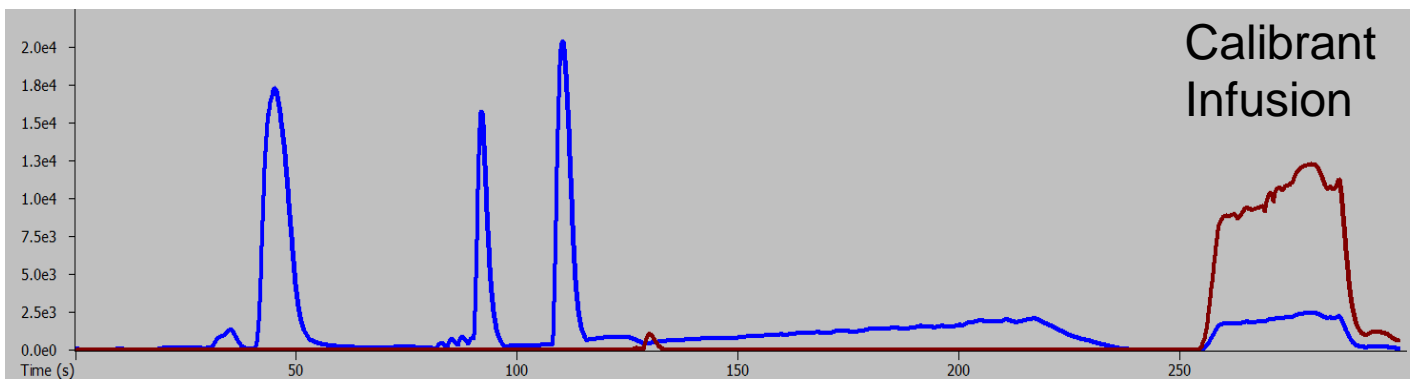
Citius[®] LC-HRT Concept



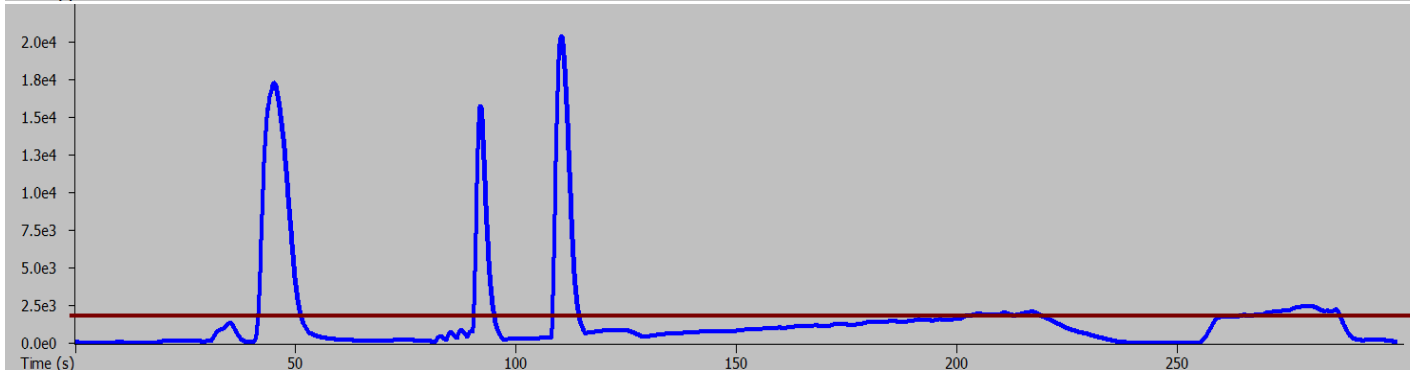
Calibration Modes for the Citius™ LC-HRT



External
Calibration

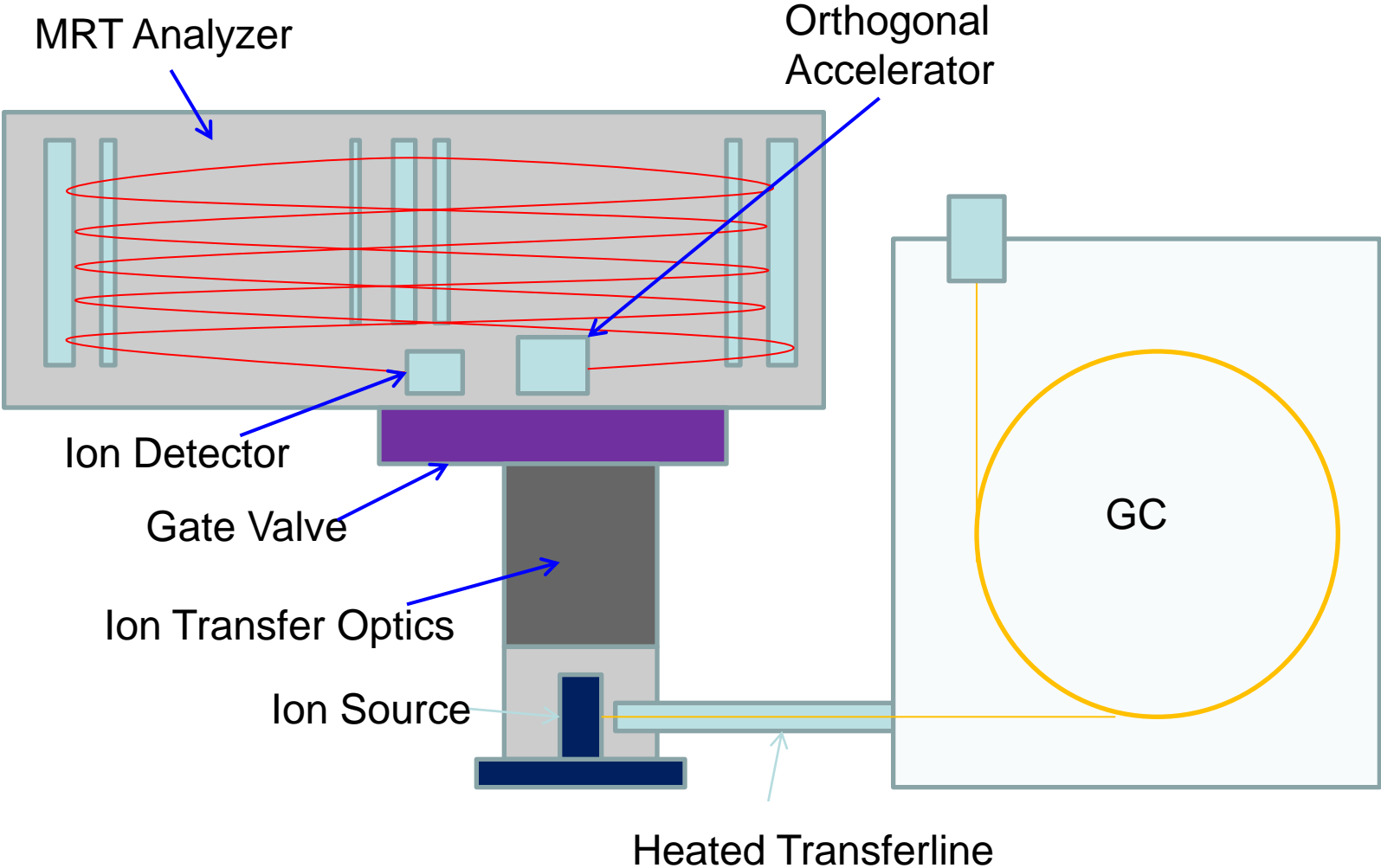


Pseudo
External
Calibration



Persistent
Internal
Calibration
“Lock Mass”

Pegasus® GC-HRT Concept



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Sample Preparation

- Materials examined and their contents:

Green Tea forms: Organic Leaf Green Tea; Green Leaf Tea; Green Tea Extract; Decaffeinated Green Tea; “Mega Tea” Green Tea (supplement containing Green Tea and Acai berry extract)

Acai Berry forms: Nature’s Bounty; Finest

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



Ginger



Acai Berries

LC-Parameter

LC: Agilent 1290 Infinity System

Column 1.9 μ m 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% B in 40 min at 0.2 mL/min

Fast Gradient 0.5 min 0% B; to 80% AcN in 3 min 0.5 mL/min

Column Temperature 50°C

Injection Volume 2.5 μ L

HRT Parameter

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 precursor/fragment)

m/z Range: 50 – 1750

m/z Calibration: External with Agilent tuning mix by co-infusion with effluent

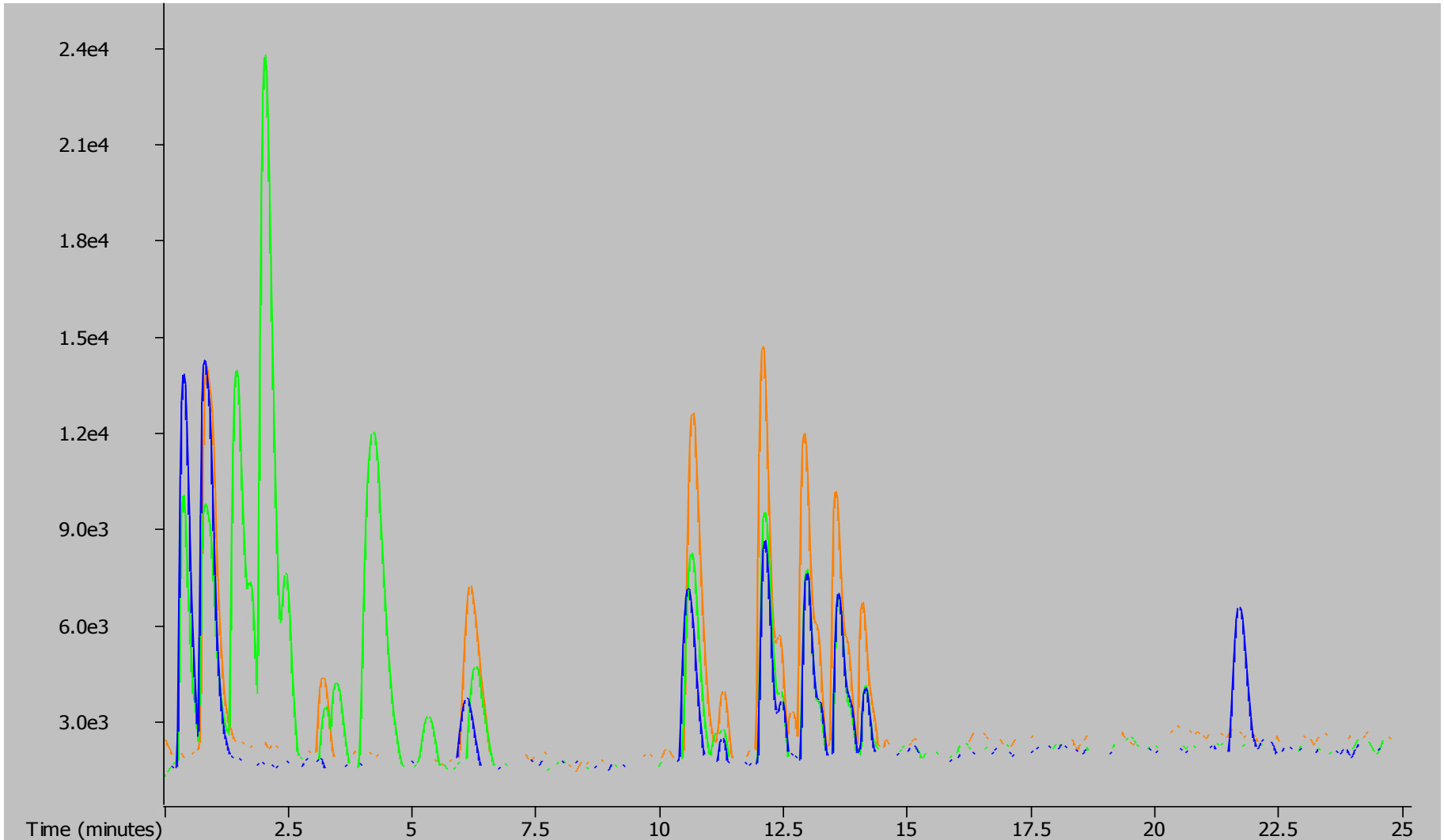
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Targeted Analytes likely to be in Green Tea, Ginger or Acai

		Monoisotopic Masses
Name	Formula	[M+H]⁺
Quinic Acid	$C_7H_{10}O_6$	191.05501
Coumaryl-quinic acid	$C_{16}H_{17}O_8$	338.09962
Gallic Acid	$C_7H_4O_5$	169.01315
Theogallin	$C_{14}H_{14}O_{10}$	343.06597
Trigalloyl-glucose	$C_{27}H_{22}O_{10}$	507.12857
Digalloyl-glucose	$C_{27}H_{22}O_{18}$	635.08789
Corilagin	$C_{20}H_{18}O_{14}$	483.07693
Epicatechin	$C_{27}H_{20}O_{18}$	633.07224
Epigallocatechin	$C_{22}H_{16}O_{10}$	441.08162
Epigallocatechin gallate	$C_{22}H_{16}O_{11}$	457.07654
Epicatechin	$C_{15}H_{12}O_6$	289.07066
Epigallocatechin	$C_{15}H_{12}O_7$	305.06558
Gallocatechin	$C_{15}H_{12}O_7$	305.06558
Kaempferol	$C_{15}H_{10}O_6$	287.05501
Quecetin	$C_{15}H_{12}O_8$	321.06049
Myrecitin	$C_{15}H_{12}O_9$	319.04484
Caffeine	$C_8H_{10}O_2N_4$	195.08765
Theobromine	$C_7H_8O_2N_4$	181.07200
Theophylline	$C_7H_8O_2N_4$	181.07200
Theamine	$C_7H_{14}N_2O_3$	175.10772

Overlay BPCs of a Green Tea, Ginger and Acai Extract (Slow Gradient)

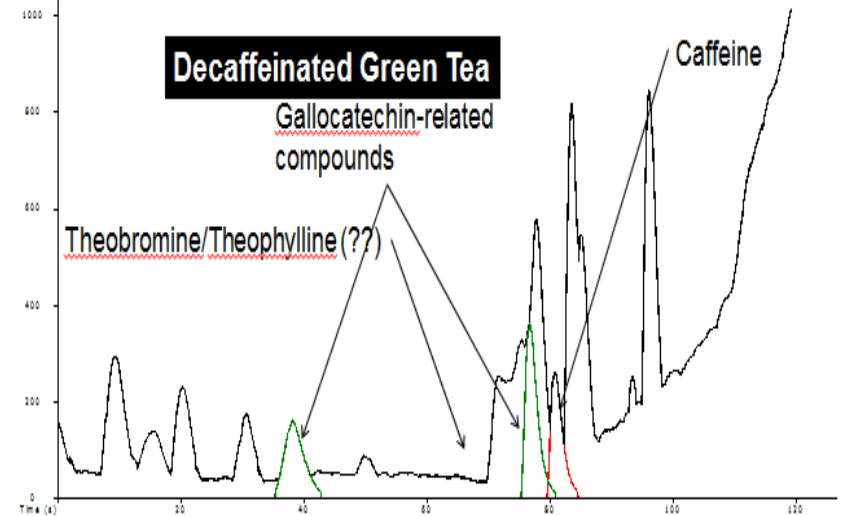
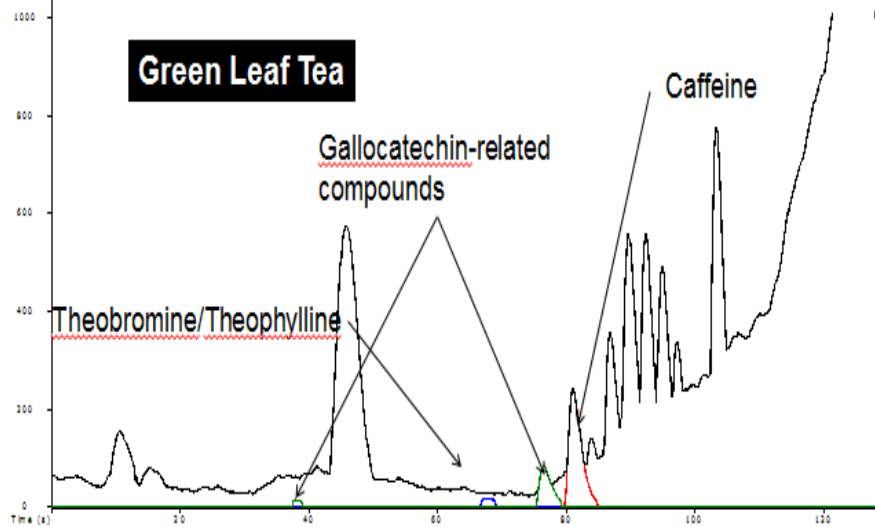
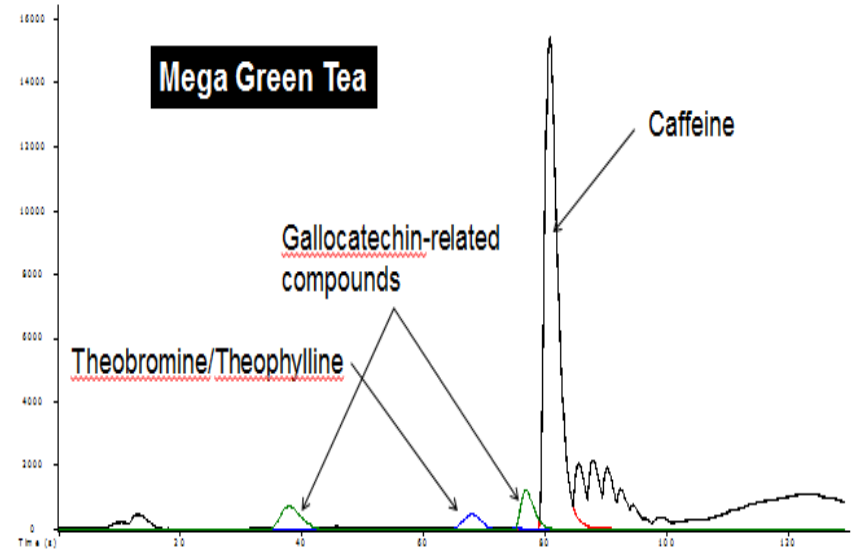
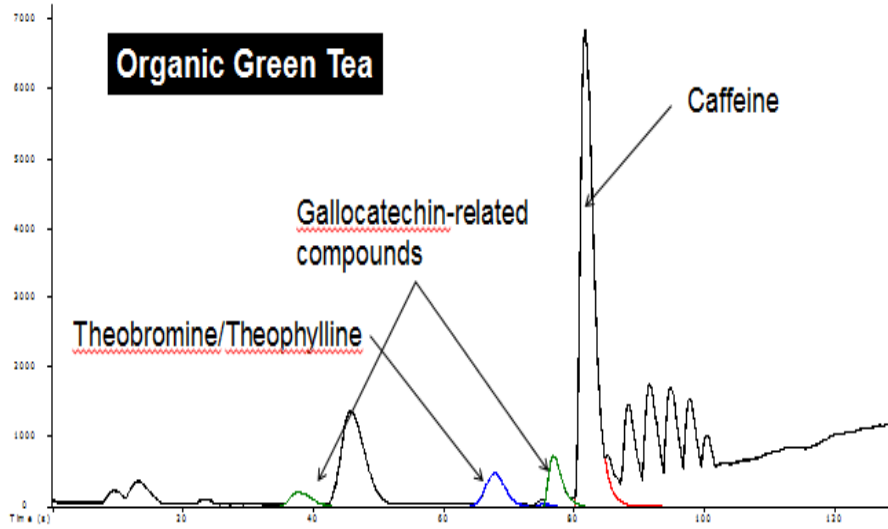


Identified Compounds in different Tea extracts

Name	expected	Green Tea		Organic		Mega Green		Decaf	
	m/z	observed m/z	m/z error in ppm	observed m/z	m/z error in ppm	observed m/z	m/z error in ppm	observed m/z	m/z error in ppm
Caffeine	195.08765	195.08753	0.7	195.08733	0.7	195.08748	1.1	195.08726	2.0
Theobromine	181.07200	181.07168	1.8	181.07211	0.6	181.07207	0.7	n.d	n.a
Theophylline	181.07200	181.07168	1.8	181.07211	0.6	181.07207	0.7	n.d	n.a
Theamine	175.10772	n.d.	n.a.	175.10766	0.3	175.10767	0.3	n.d	n.a
Epigallocatechin	305.06558	305.06502	1.8	305.06568	0.4	305.06532	0.9	305.06587	1.1
Ephedrine-like	152.07061	152.07041	1.3	n.d.	n.a	152.07046	1.0	152.07032	1.7

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

UHPLC Analysis of different Tea extracts



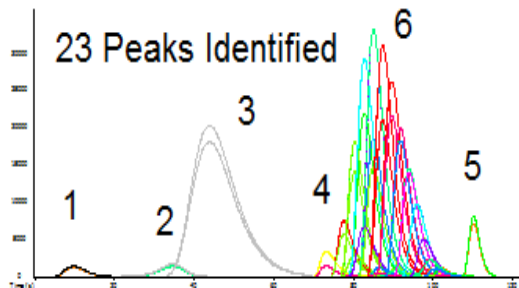
Different Dosage Forms

Active ingredient per dosage (mg)

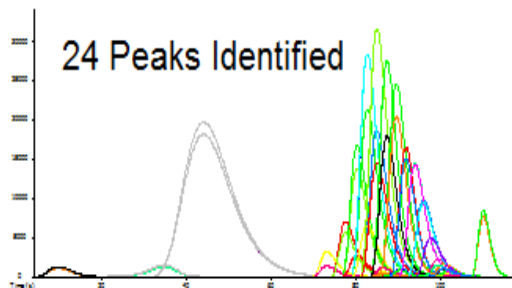
	Dosage	Acetaminophen M+H ⁺ = 152.0706	Dextromethorphan M+H ⁺ = 272.2014	Guaifenesin M+H ⁺ = 199.2235	Phenylephrine M+H ⁺ = 152.0706
caplets	Generic	325	10	200	5
caplets	Innovator	325	10	200	5
gels	Generic	325	10	0	5
gels	Innovator	325	10	0	5
liquid	Generic	325	10	0	5
liquid	Innovator	325	10	0	5

Acquisition Speed and Characterization of the Dosage Form

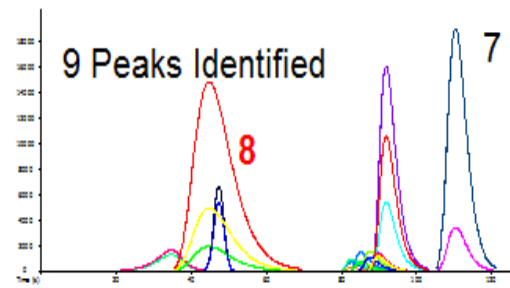
TOP – Selected ion traces from the UHPLC Analysis (0.25 ml/min) using 40 spectra/sec acquisition (20 “Parent ion” spectra/sec and 20 “Fragment ion”)



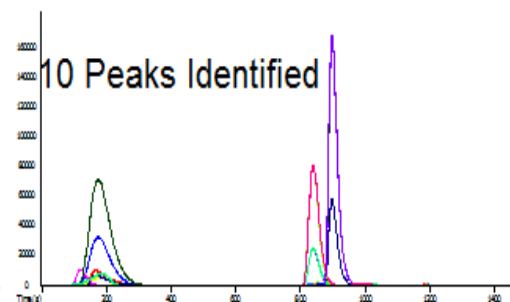
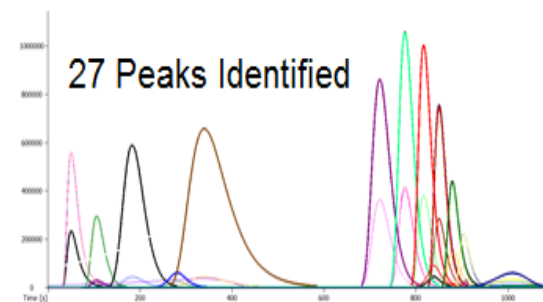
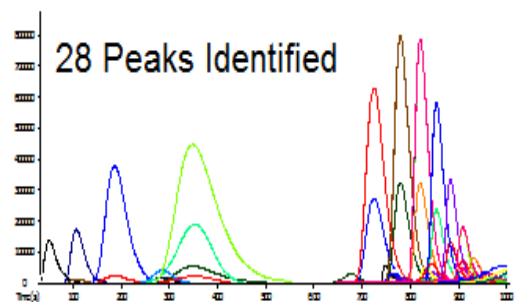
Analysis of innovator gels



Analysis of generic gels

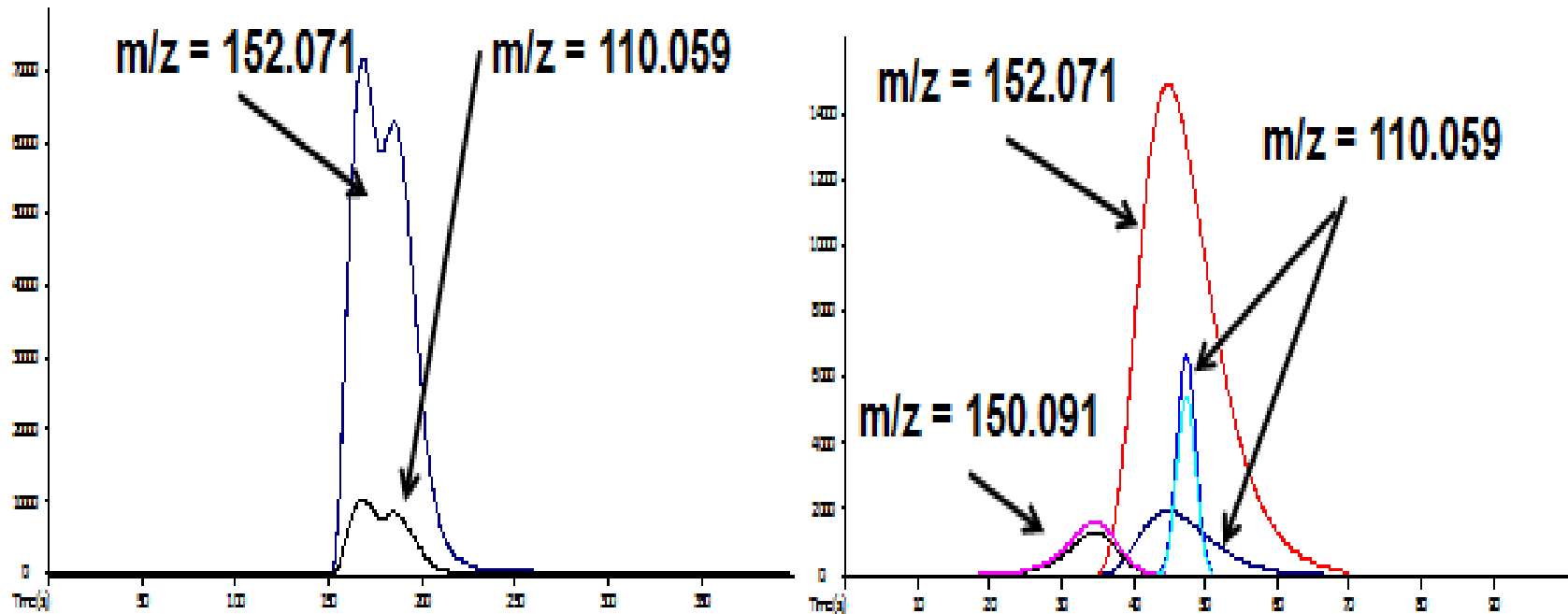


Analysis of Innovator caplets



BOTTOM - Selected ion traces from the HPLC Analysis (0.10 ml/min) using 4 spectra/sec acquisition (2 “Parent ion” spectra/sec and 2 “Fragment ion”)

Extraction of a coeluting Impurity



Extracted Ion Chromatogram of $m/z = 110.06$ and 152.07 in HPLC and UHPLC Analyses of innovator caplets. ($m/z = 150.091$ is also included in the UHPLC analysis for reference)

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Summary and Conclusions

- High performance mass spectrometry has been used to differentiate various forms of green tea, ginger and acai berry extracts with respect to their “nutrient” content.
- Identification was achieved using accurate mass determination with measured mass accuracies of typically less than 1ppm
- In addition relative isotope abundance (RIA) and fragment ion information was obtained to facilitate identification of analytes.
- High speed UHPLC analysis was achieved using fast spectral acquisition (40 spectra/sec), which also permitted the identification of a co-eluting degradant.

Thank You For Your Attention



For more information:
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