Plant-Climate Interaction Effects on the Tea Metabolome

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5th SBSE International Meeting
September, 23-24, 2019
Paris France

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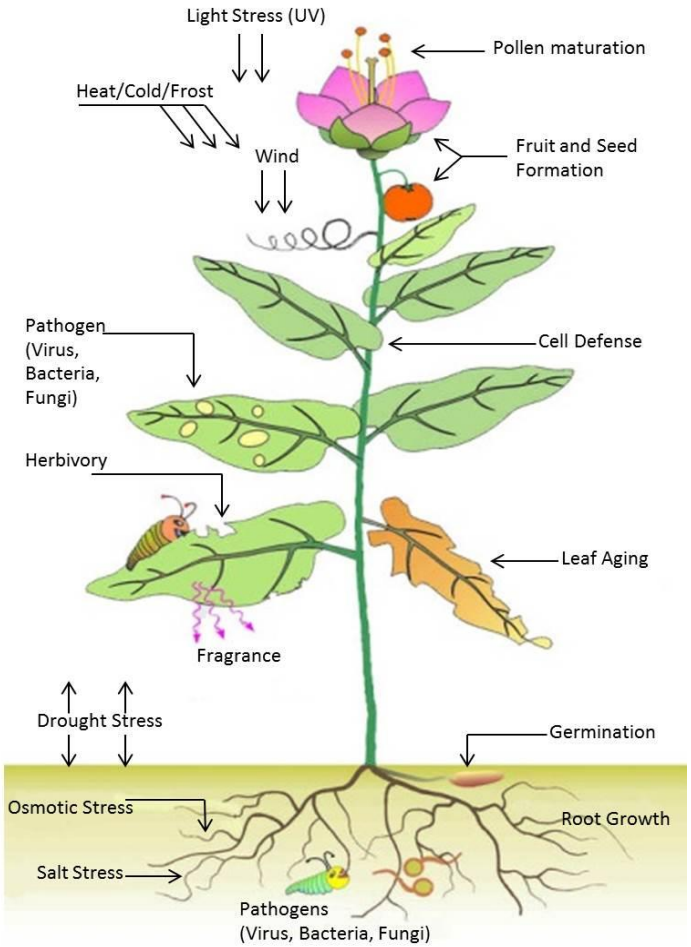
Plant-Climate Interactions

- Changes in climate are predicted to have large impacts on crop production
  - Direct impacts include shifting weather patterns (e.g., some areas are becoming warmer, others colder), with extreme weather events increasing in frequency/severity
  - Indirect impacts include increasing stress due to pests and pathogens
- Climate effects on yield are well-studied
- In contrast, little is known about climate effects on crop quality
  - Our objective is to investigate plant-climate interactions and their effect on flavor and nutritional quality
  - To investigate these issues we combine technologies sold by Gerstel, Agilent, and Ion Analytics to obtain data on how changes in weather affect the total, detectable volatile metabolite profile in tea plants



Leibniz Institute of Plant Biochemistry, 2013

How Do Abiotic and Biotic Pressures Cause Changes in Metabolite Distribution & Concentration in Plants

- Primary metabolites provide essential functions for growth and development, including proteins, amino acids, sugars, carbohydrates, vitamins, etc.
- Secondary metabolites provide specific functions for pollination and defense, including polyphenolics, tannins, terpenoids, alkaloids, steroids, aromatics, etc.
- Primary metabolites are typically associated with taste; secondary metabolites with flavor.
- Nutritional benefits are obtained from both primary and secondary metabolites.

Objective and Approach

Stir Bar Sorptive Extraction

Direct Contact Sorptive Extraction

- Our objective in this study is to learn how extreme and daily changes in weather impact the sensory and nutritional quality of tea from Yunnan and Fujian Provinces
- Our approach is to use Twisters to extract organics from leaves in the field (direct contact) and lab (liquid infusion)
- After collection, leaves were immediately microwaved in the field to stop enzymatic oxidation.
  - We consider these samples "environmental," representing the total detectable, volatile metabolome
  - We typically detect 450 compounds in infused leaves (90 °C); 150 compounds by direct contact analysis (20-28 °C)

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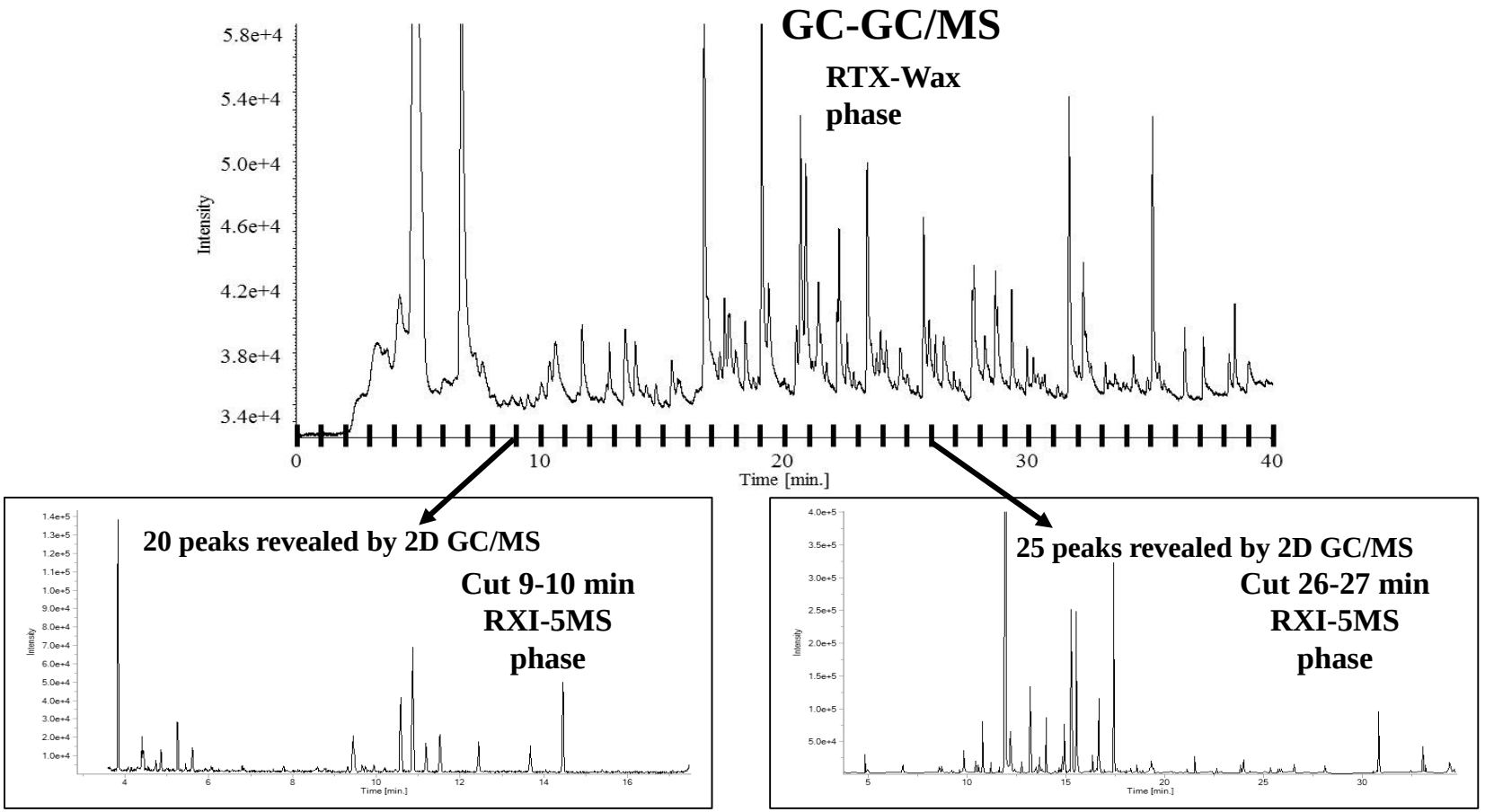
Optimizing MS Workflows

All analyses are carried out on a 1D / 2D GC/MS, with olfactometry detection

All data analysis is carried out using the Ion Analytics (IA) software to create matrix-specific databases and to quantify metabolite concentrations

Ion Analytics Creates Databases from 1D / 2D GC/MS Data



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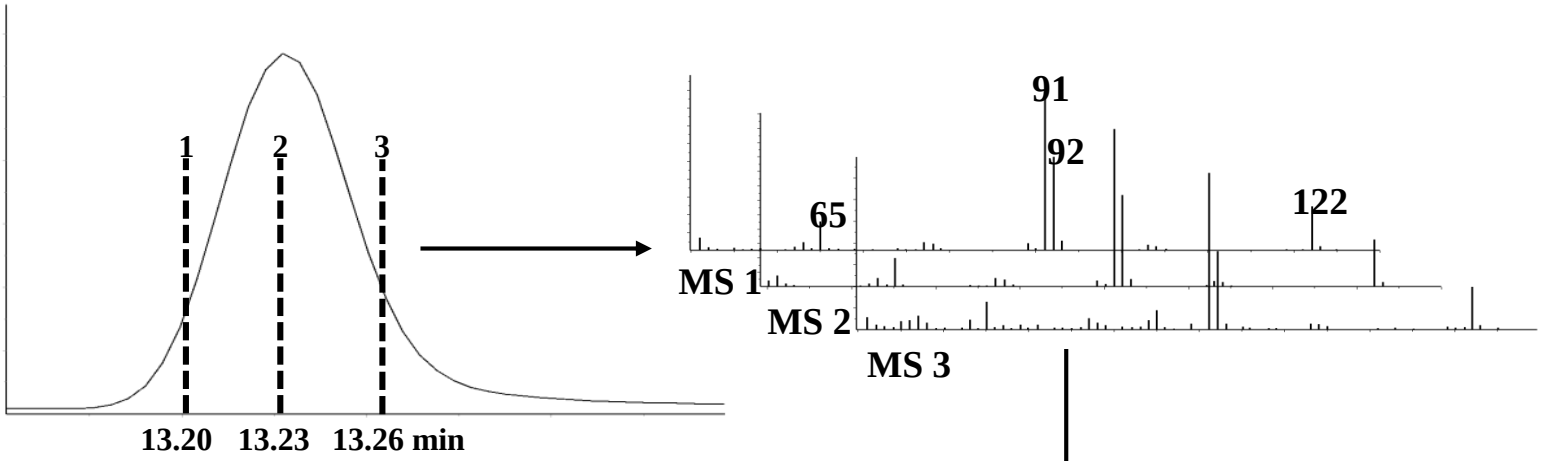
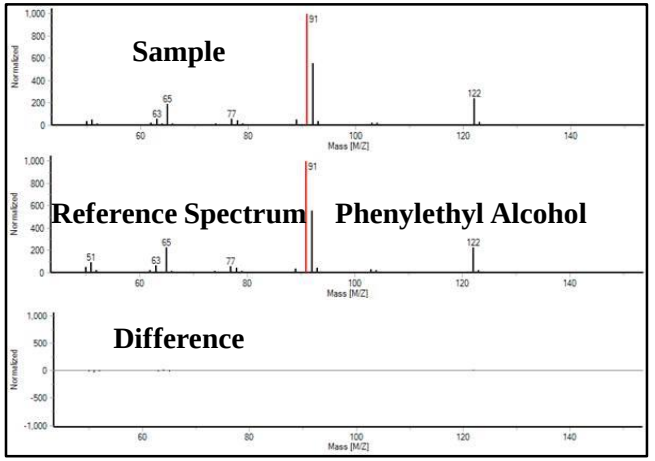
Ion Analytics Improves MS Workflow Efficiency

Automatically identifies and creates compound libraries



in example 1,
compound identification is straightforward when there is no matrix interference from other compounds in the sample



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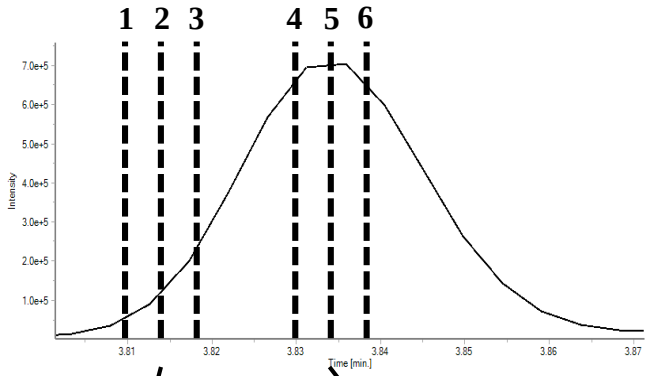
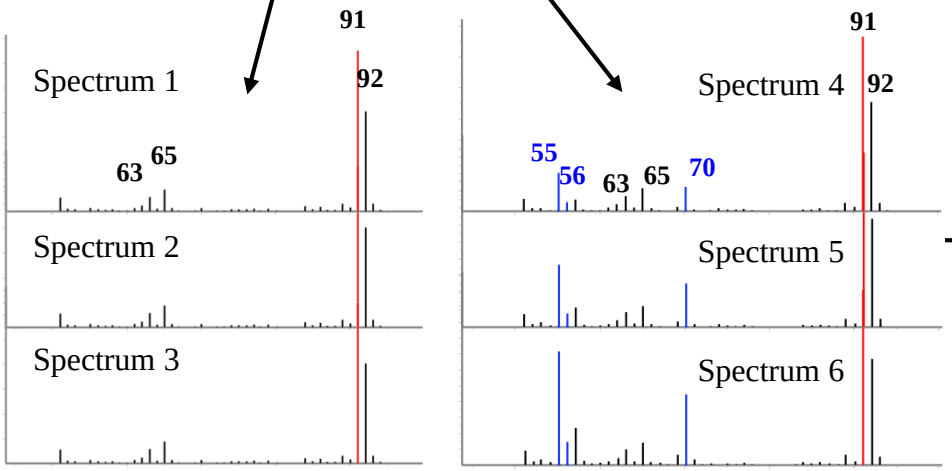
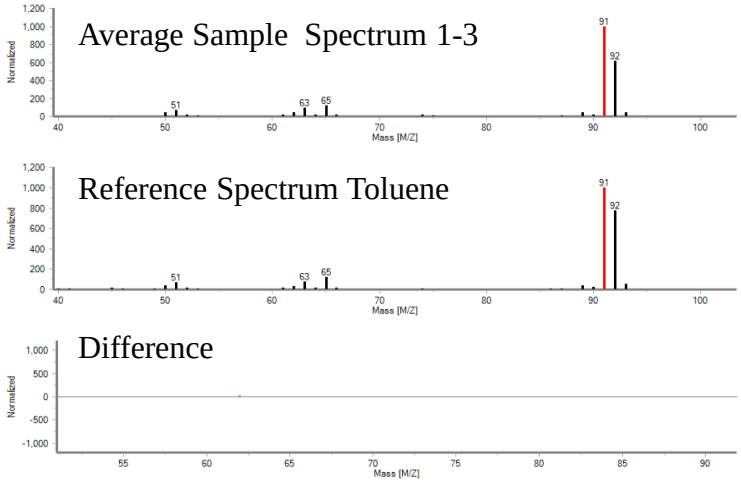
MS Subtraction – Target 1 (Toluene)



in example 2,
although 3 compounds coelute, the IA software systematically identifies each compound by first identifying and subtracting target compound spectra (in this case, toluene) from ion signals at each peak scan





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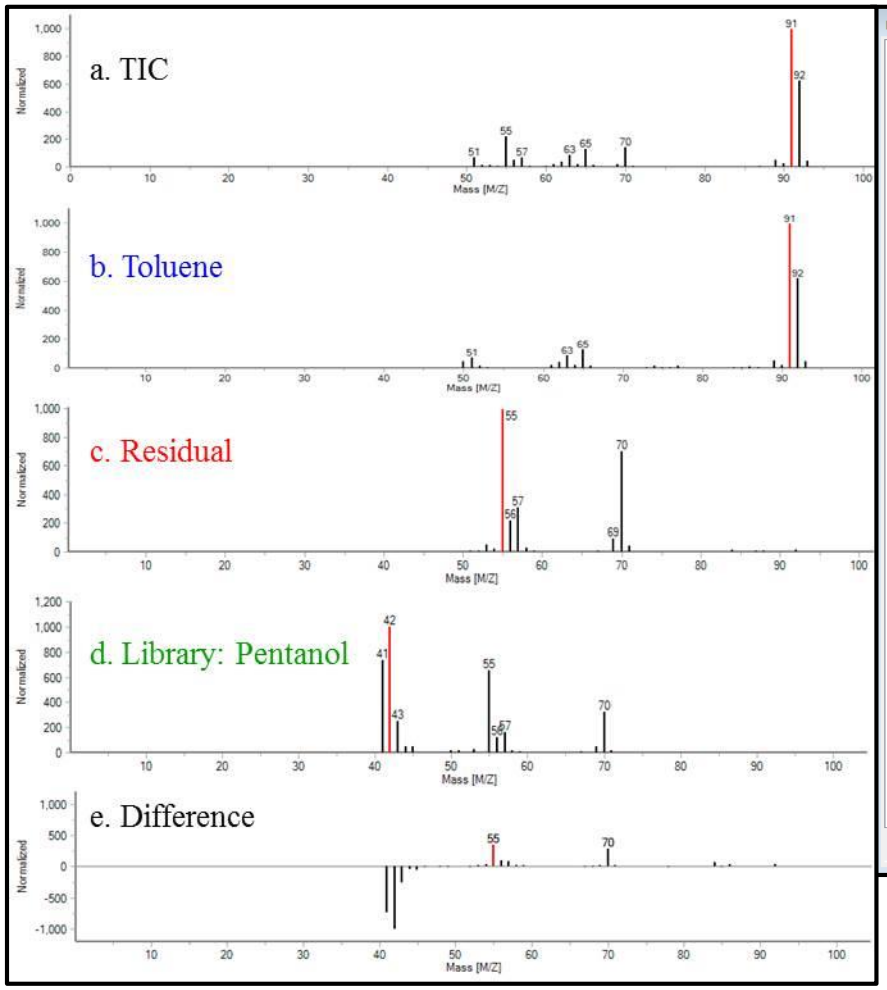
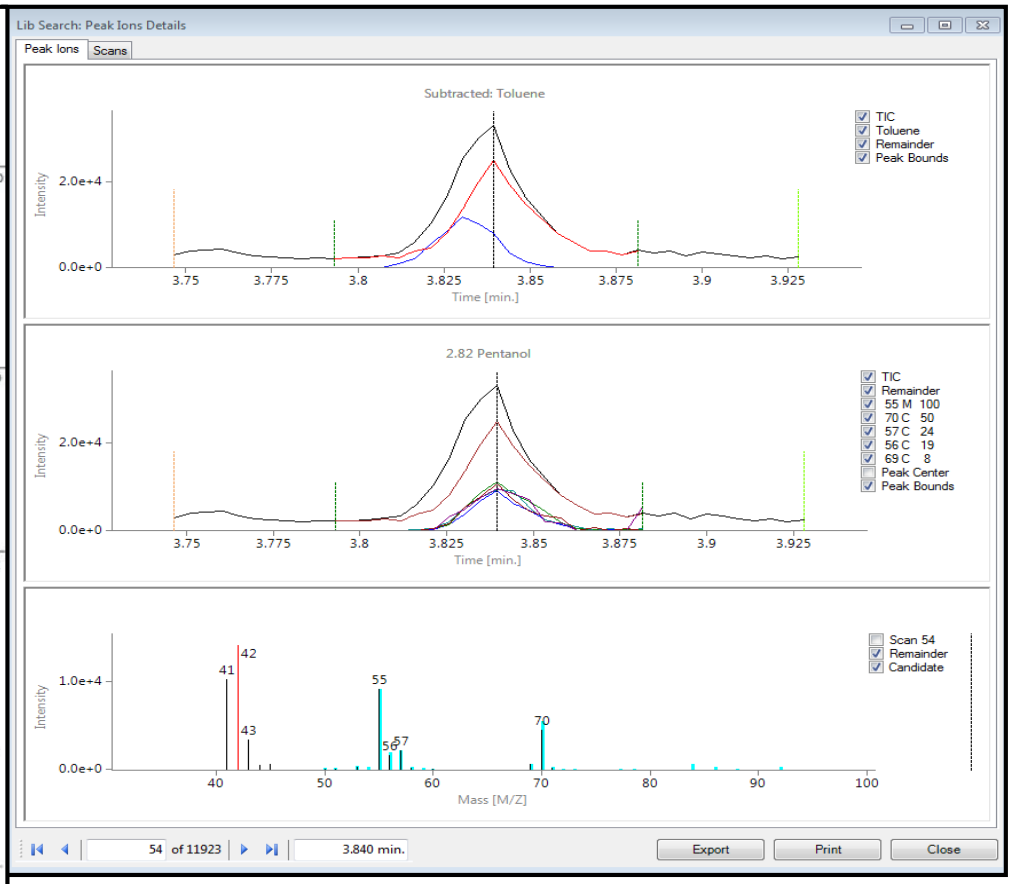
MS Subtraction – Non Target 1 (Pentanol)





MS subtraction of the toluene yields residual spectrum (c), which matches pentanol in NIST, Wiley, Adams, etc.

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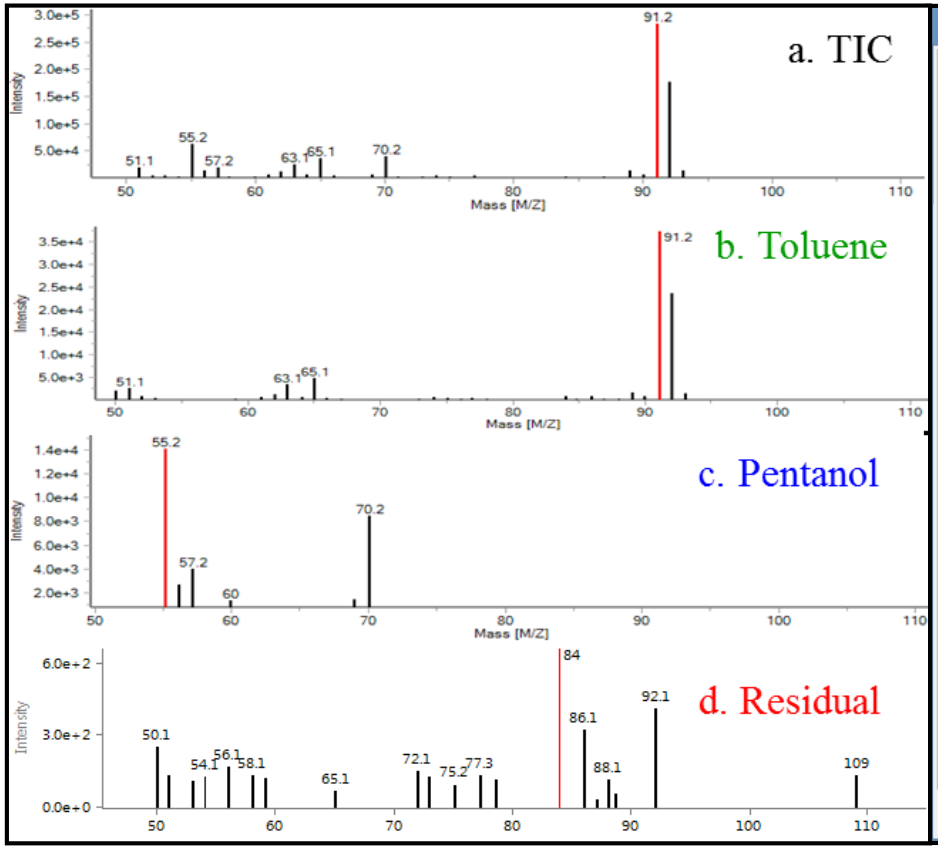
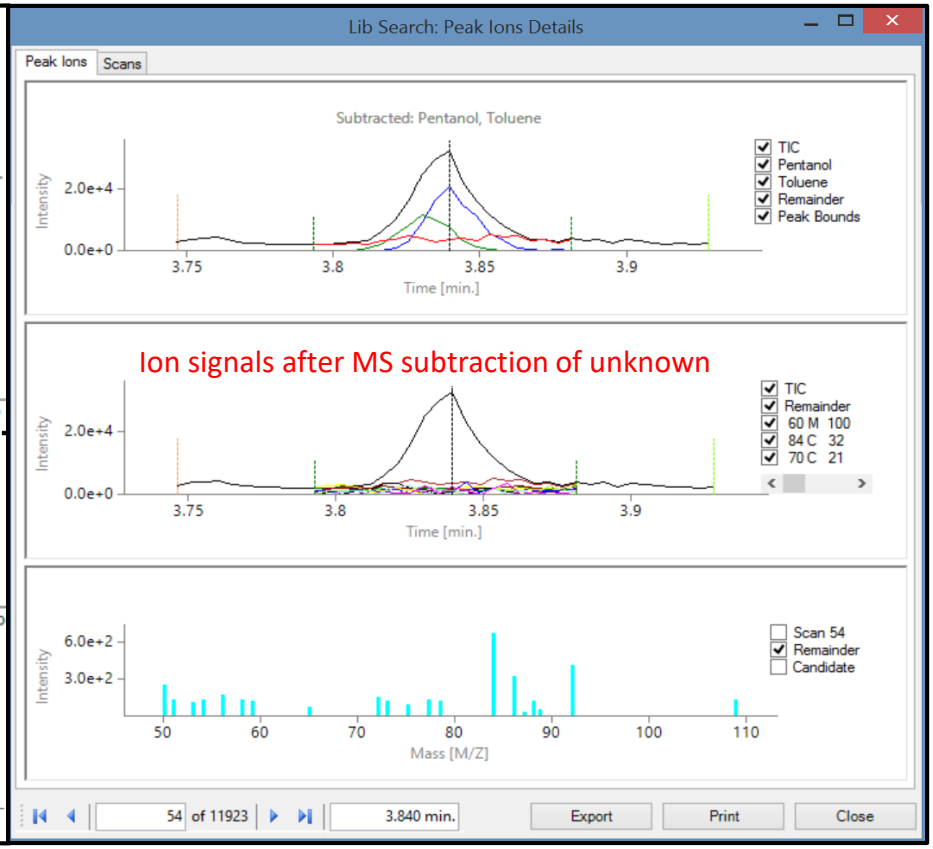
MS Subtraction – Non Target 2 (unknown)





- MS subtraction of pentanol yields residual spectrum (d). Since the spectrum is not in any commercial library/database or literature, we assign it a numerical identifier in the IA database, making it a target compound in subsequent analyses
- MS subtraction of the unknown results in ion signals that approximate background signals

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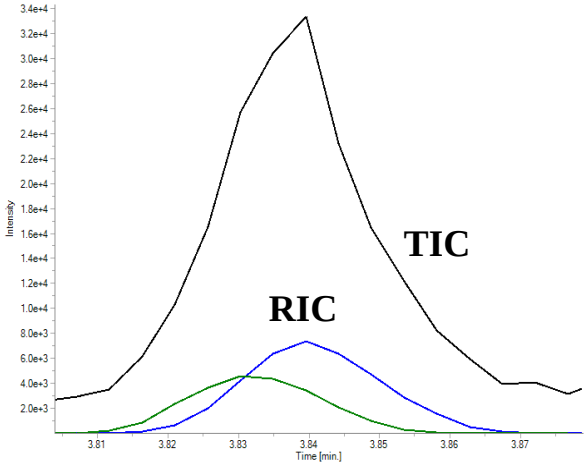
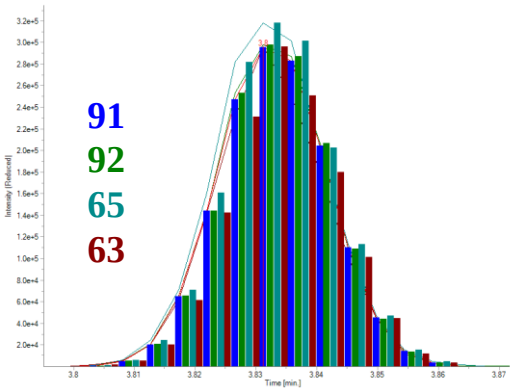
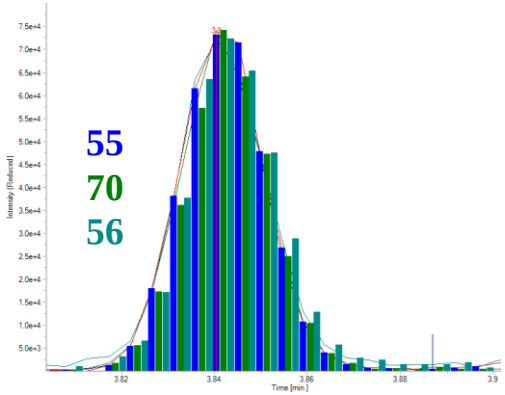
Target Compounds Identified by Spectral Deconvolution





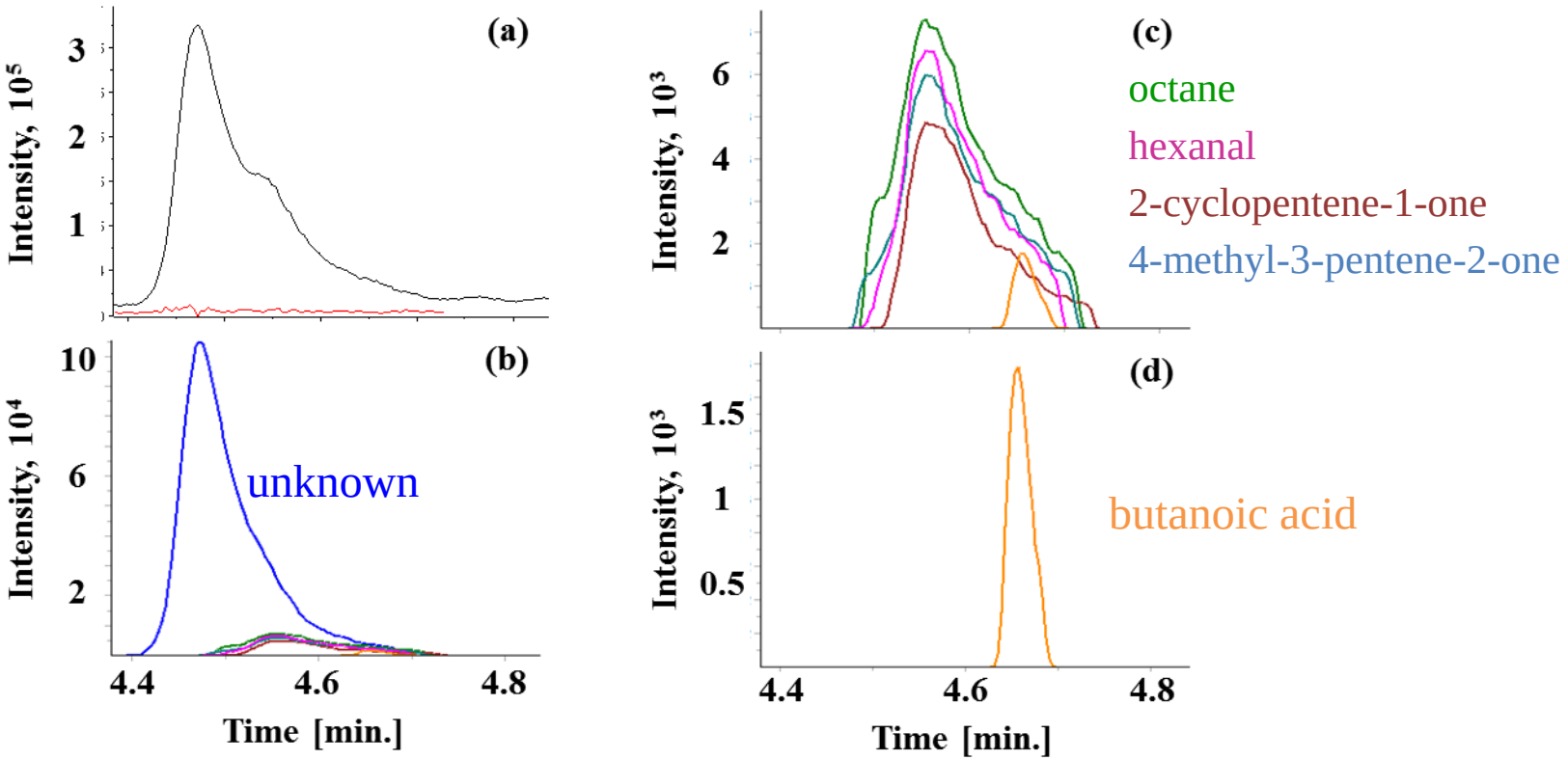
Toluene



n-Pentanol

- Once the library/database is created, we use GC/MS and the IA software to identify and quantify metabolites no matter sample complexity
- Compound identification is made when ion signals fall within the acceptance criteria set by analyst

MS Workflow - Target/Nontarget Analysis



(a) TIC peak (black). (b) RIC chromatograms of target and nontarget compounds.

(c) RIC peaks after subtraction of blue peak mass spectrum from the TIC, identification by 2D GC/MS after reversing the columns.

(d) RIC peak after spectra subtraction of the 4 peaks in (c).

(a) Background signal (red) after MS subtraction of all compounds from the TIC.

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How Changes in Yunnan and Fujian Weather Conditions Affect Tea Quality

Although Yunnan and Fujian are impacted by the East Asian Monsoon Rains, Yunnan experiences drought conditions in the spring, Fujian does not

The East Asian Monsoons are arriving earlier, lasting longer and are beginning to affect tea production and quality

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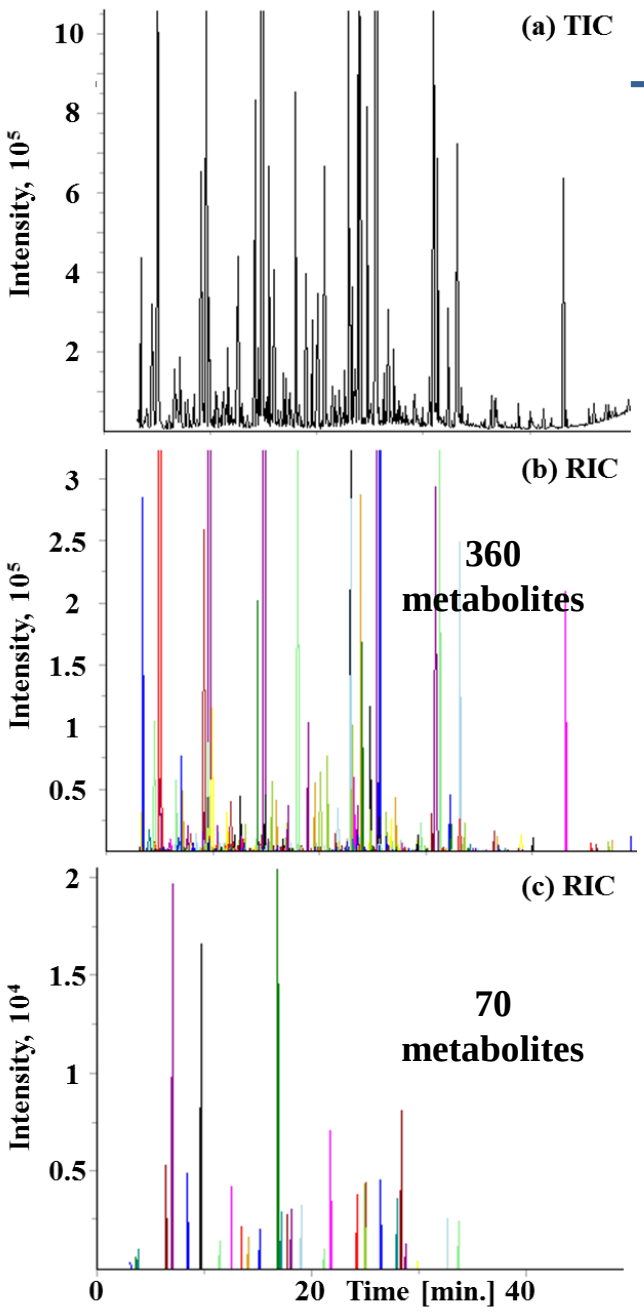
Spring Tea from Fujian



Target Compound Analysis:

- GC-GC/MS was used to create the initial (Yunnan) tea database
- We found 360 common compounds in Fujian tea, after spectral deconvolution of target compounds (Yunnan database) from GC/MS TIC (a)

Nontarget Compound Analysis:

- After MS subtraction of targets, we identified 70 metabolites (non-targets, c)
- 430 analytes quantified and tracked

Target/Nontarget Analysis:

- Analysis of spring and summer samples at high and low elevations from Yunnan and Fujian over a 3-year period has produced a tea database that consists of 750 metabolites; all caused by various stress factors induced by changes in weather over time

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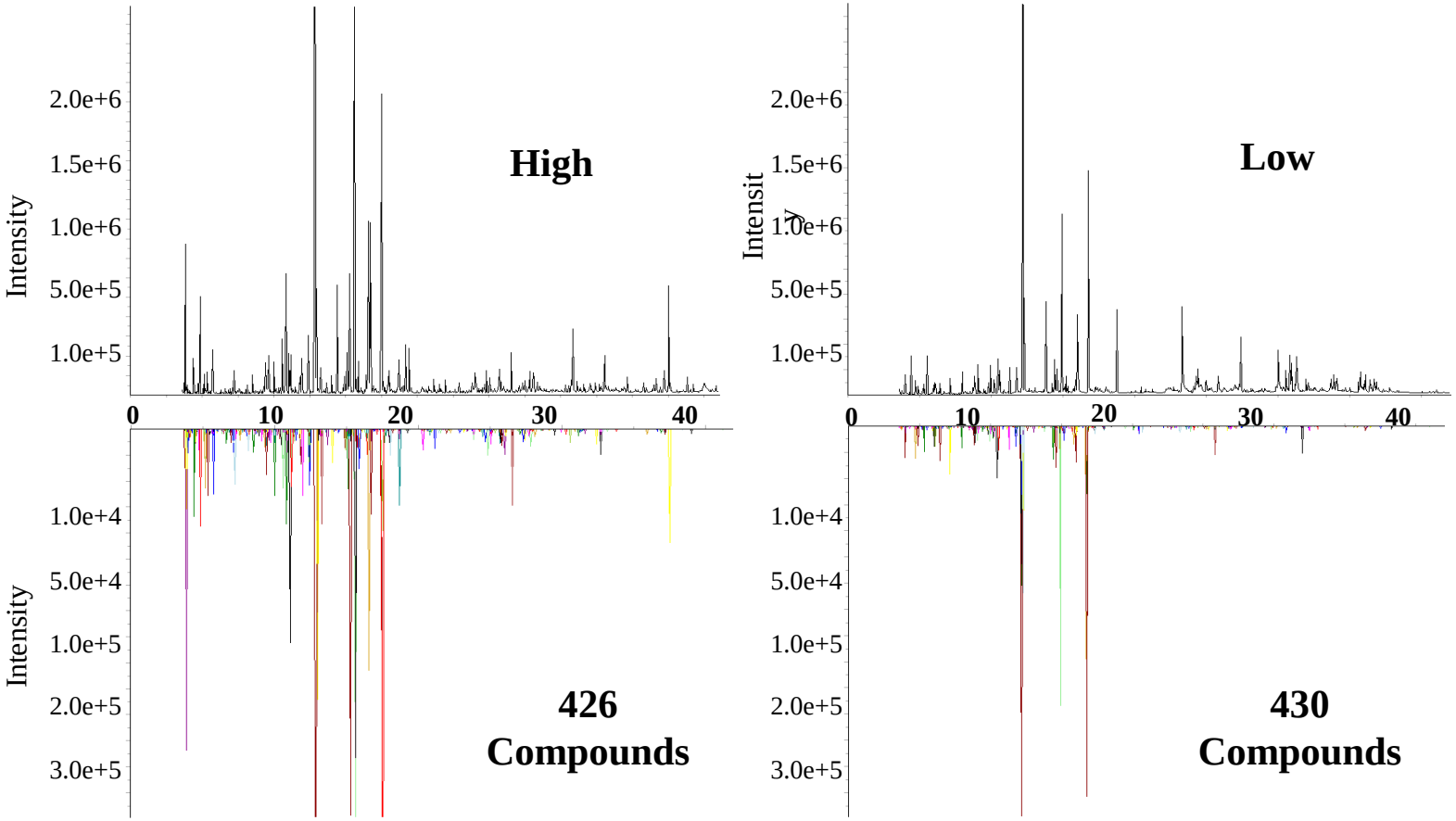
Differences in High and Low Elevation Teas

using elevation as a surrogate for temperature, 5 °C



Although the number of compounds detected are about the same, metabolite distribution and concentration are very different

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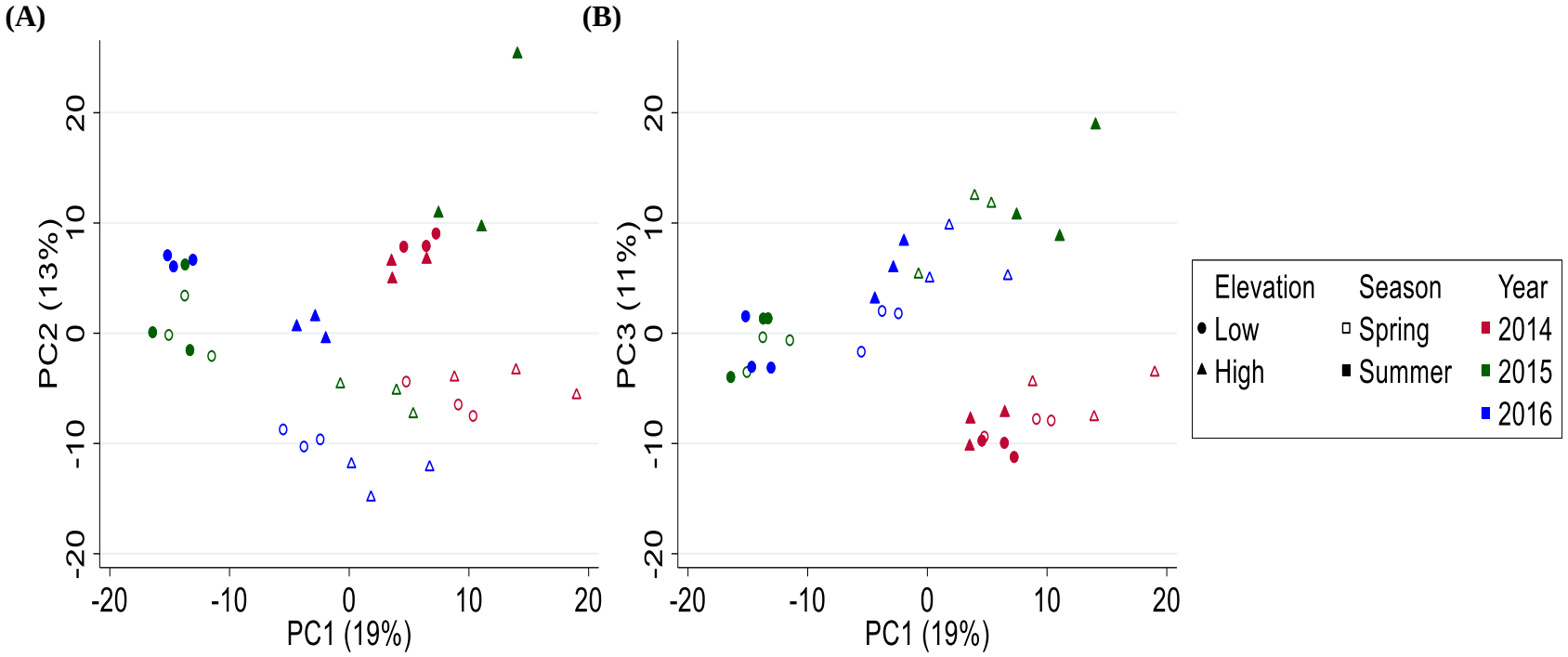
Changes in Metabolite Distribution and Concentration in Yunnan over a 3-year Period



- Metabolites differ by elevation (circles vs. triangles) on PC1 (except for 2014), season (open vs. closed shapes) on PC2 and year, 2014 (red) vs. 2015/2016 (green/blue) on PC3
- 3-way PERMANOVA confirmed findings, with significant main effects due to elevation ($F_{(1,24)}$ = 34.57, p = 0.001), season ($F_{(1,24)}$ = 11.23, p = 0.001), and year ($F_{(2,24)}$ = 13.05, p = 0.001)
- Significant interactive effects found between year and elevation ($F_{(2,24)}$ = 13.29, p = 0.001), year and season ($F_{(2,24)}$ = 4.01, p = 0.010), but not season and elevation ($F_{(2,24)}$ = 0.78, p = 0.425)

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Small Differences in Temperature Causes Big Changes in Chemistry, statistically important metabolites in high elevation tea

Compound	VIP	p-value	% Diff.	Aroma	Health Property
High Elevation					
1-Ethyl-1H-pyrrole-2-carboxaldehyde	2.479	0.0002	51	roasted, smoky	
m-Xylene	2.260	0.001	49	plastic	
p-Xylene	2.254	0.001	48	sweet, grain	
2-Cyclohexen-1-ol	2.005	0.005	-	caramelized, floral	
Benzeneacetonitrile	1.960	0.0008	50	floral	
(*Z*)-Jasmone	1.943	0.0002	60	jasmine, floral	antibacterial anticancer
α-Ionene	1.930	0.02	48	floral, violet	
(2*E*)-Hexenol	1.928	0.005	72	green, leafy, fruity	
7	1.888	0.01	37		
(*E*)-Caryophyllene	1.885	0.008	47	green, spicy, woody	analgesic antianxiety antidepressant anticancer anti-inflammatory antioxidant
2-Acetylfuran	1.808	0.01	43	sweet, balsamic	

Continuation, high elevation tea

(3Z)-Hexenol	1.778	0.02	26	green, grassy	anti-fatigue anti-nociceptive antifungal anti-stress
70	1.654	0.02	53		
6	1.625	0.03	-		
Manool	1.589	0.03	59		antibacterial anticancer antifungal anti-inflammatory
1	1.560	0.05	30		
5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	1.558	0.03	32		
α-Calacorene	1.537	0.02	58	woody	antibacterial antioxidant
1-Ethyl-1H-pyrrole	1.514	0.04	75	burnt	
Cadalene	1.498	0.03	71		antibacterial antioxidant
Theaspirane	1.347	0.02	44	tea, herbal, honey	
27	1.328	0.02	27		
50	1.320	0.04	43		

Small Differences in Temperature Causes Big Changes in Chemistry, statistically important metabolites in low elevation tea

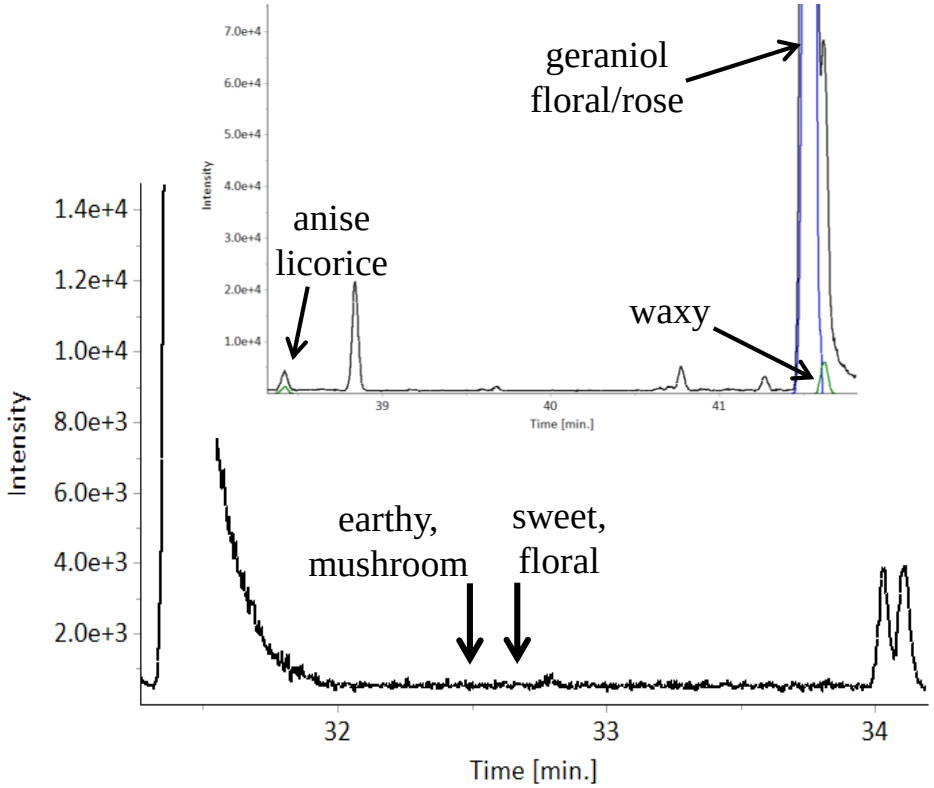
2,6-Dimethyl-3,7-octadiene-2,6-diol	2.255	0.001	-	fruity, herbal	
66	1.906	0.01	-86		
25	1.674	0.01	-112		
trans-Linalool oxide (pyranoid)	1.620	0.02	-84	woody, fresh	
Cyclohexanone	1.590	0.02	-95	minty	
72	1.509	0.01	-		
Isovaleric acid	1.269	0.05	-123	cheesy, rancid	
2,5-bis(1,1-dimethylethyl)phenol	1.448	0.03	-157		
Dihydroactinidiolide	1.398	0.02	-96	fruity, woody	
30	1.398	0.02	-323		
2,2-Dimethoxy-1,2-diphenyl-ethanone	1.375	0.01	-		
6-Azabicyclo[3.2.1]octane	1.256	0.02	-93		
46	1.217	0.008	-199		
2,3-Dihydrobenzofuran	1.091	0.02	-154	green, herbal	

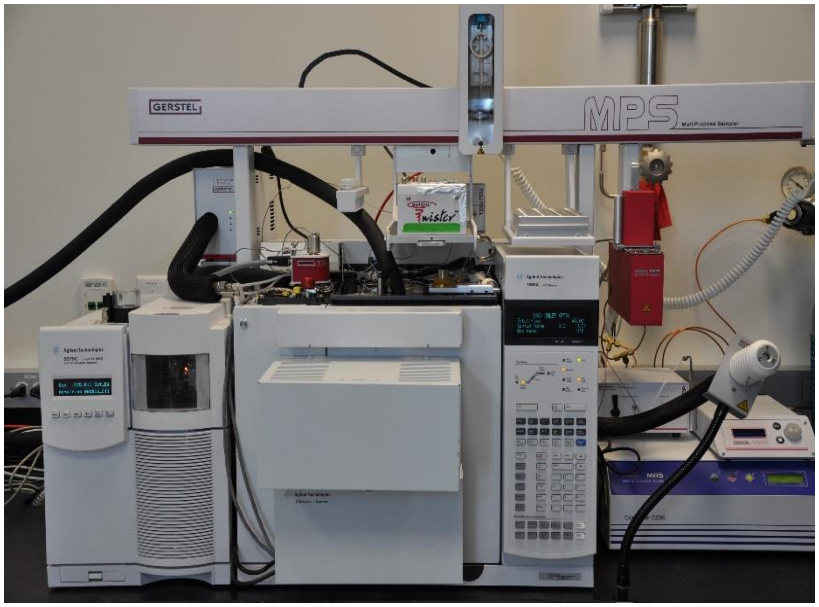
Yunnan – Catechins (antioxidants)

Compound	Spring vs Summer Δ (mg/g) ± SE	p value
Catechin	2.1571 ± 0.7766	0.0083
Epigallocatechin	17.2822 ± 4.6412	0.0006
Epigallocatechin 3-gallate	14.9912 ± 3.8607	0.0004
Gallocatechin 3-gallate	2.3890 ± 0.5856	0.0002
Epicatechin 3-gallate	8.9411 ± 3.0939	0.0062
Gallic acid	1.6724 ± 0.3957	0.0001
Caffeine	11.5157 ± 2.8439	0.0002
Total phenolic content	-5.8042 ± 2.2565	0.0139
Antioxidant capacity	-16.7845 ± 2.1730	<0.0001

Although catechin concentrations are higher in spring (drought) tea,
total polyphenolics and antioxidant potential are higher in summer (monsoon) tea

1D / 2D GC/MS Olfactometry Detection of Sensory Active Compounds





- Non-detects can be quantified by using Twister to increase mass on-column
- IA subtracts target compound spectra of coeluting compounds to identify unknowns masked by the matrix

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Conclusions

- Twister vs. SPME yielded 450 vs. 300 metabolites in tea compared to 150 by direct contact
- Ion Analytics reduces the data processing time of GC-GC/MS (50 data files) from months to weeks and, from days to < 2 hours of target/untargeted GC/MS analysis
- Employing untargeted/targeted analysis and integrating results with GC-O, sensory, and other analyses provides both detailed understanding of how plants react to a variety of stress factors
- Moderate temperature, extreme rainfall, and herbivory differences cause striking differences in the concentration of sensory and nutritional compounds in tea

The combination of technologies provides unmatched ability to detect and identify metabolite distributions and concentrations in complex samples such as tea. They also provide the means to unmask off-notes and flavors as well as quantify environmental pollutants in difficult to analyze samples.

Ion Analytics Highlights

(A) Total ion current (TIC) chromatogram of high elevation spring tea from Fujian, China

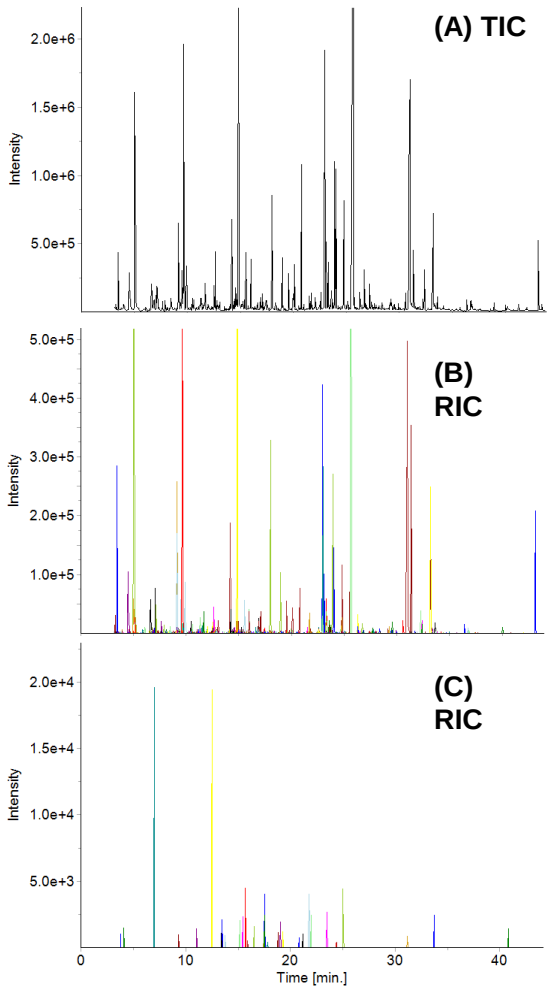
(B) Using the 600 compound database created by analyzing samples from Yunnan, China, found 444 common (target) compounds – spectral deconvolution

(C) After subtracting the mass spectra of the 444 target compounds from the TIC chromatogram, the reconstructed ion current chromatogram reveals 32 new (untargeted) compounds – MS subtraction

After subtracting the mass spectra of the untargeted compounds, the residual signal equals baseline

Every detectable compound is positively (standards) or tentatively (library MS and RI match) identified or assigned a numerical identifier and then added to the database

The tea database has MS and RI data for 750 compounds



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Target/Non-target MS Workflows

The mass spectra (b) across the peak (a) are different due to coeluting compounds

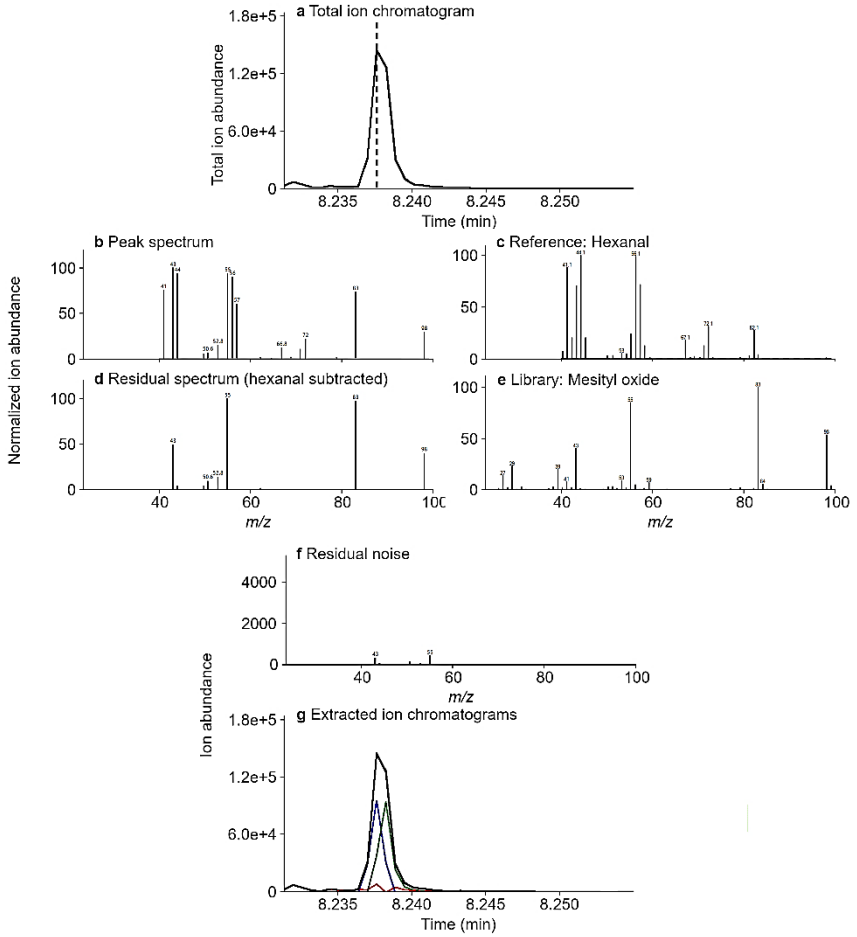
Target compound analysis using the tea database identifies hexanal in the sample

After subtracting the mass spectrum of hexanal (c) from the TIC, the residual spectrum is shown in (d)

Sample (d) and library (e, database) spectra match mesityl oxide, which was confirmed by matching the MS and RI of mesityl oxide

Subtraction of the mesityl oxide spectra yielded instrument noise (f) and baseline signal (g, red line)

The RIC chromatograms for hexanal (blue) and mesityl oxide (green) are shown in (g).



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Currently Available Databases

Matrix-specific Databases (compounds)				
Coffee	1250		Tea	750
Raspberry, Blackberry, Strawberry	900		Sweet orange	500
Lemon	450		Bitter orange	350
Juniper berry	400		Cheese/Fermentation volatiles	400+
Unified Database (2500 compounds)				

- When we analyze complex samples such as plant materials by GC/MS , more than 60% of the metabolites can be identified by target analysis (spectral deconvolution); the remainder by non-target analysis (MS subtraction)
- The database grows by adding new compounds (non-targets) from subsequent analyses
- Once analyzed, we use commercial libraries and literature to identify "potential" sensory active compounds in the sample. Since we know retention times, we go right to that point (time) in the chromatogram to detect odor by 1D/2D GC/MS
- If coelution exists, GC-GC/MS-O cuts that portion of the sample from the 1st to the 2nd column, with detection by MS and olfactory
- Since sensory active compounds are concentration dependent, we determine actual aroma in the sample. If the literature says compound smells like rose, it may not due to its concentration in the sample. 1D /2D GC/MS-O helps to identify synergistic and masking effects
- If needed to problem-solve, the ODP4 can be used to collect sensory active compounds we detect but can't identify for analysis by IR, UV, NMR, etc.
- The combination of Gerstel and Ion Analytics technologies are powerful tools in solving complex sensory issues