



WORLD BREWING CONGRESS

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#ElevateBeer



Gas Chromatography with Time-of-Flight Mass Spectrometry to Monitor the Aging Process in Beer

Elizabeth Humston-Fulmer and Joe Binkley

Life Science and Chemical Analysis Centre

LECO Corporation, St. Joseph, MI





Outline

Objective: Investigate chemical changes related to the aging process of beer using non-targeted discovery based tools

- Analytical tools
- Target analysis of analytes of known interest
- Discovery analysis with non-targeted approach





Analytical Approach

- Targeted screening approaches may not give a complete picture
- For discovery, non-targeted analysis to separate components within a complex mixture and identify expected and unexpected analytes

GC-TOFMS

- Gas chromatography (GC) to separate individual analytes from complex matrix
- Time-of-flight mass spectral (TOFMS) detection for identification & quantitation
- Mathematical deconvolution to further isolate individual analytes



LECO's Pegasus HT



Experimental Design: Aged Beer Analysis

- *Samples:* Commercially available canned IPA purchased from grocery store
- *Sample prep:* Accelerated/simulated aging with elevated temperature^[1]

Sample		Accelerated Aging (days at 40°C = months added)	Months Added
A	Packaging to purchase = 6 weeks		0
B		1	1
C		2	2
D		3	3
E		4	4
F		5	5
G		6	6
H		8	8
I		12	12

Packaging to analysis = 2 months

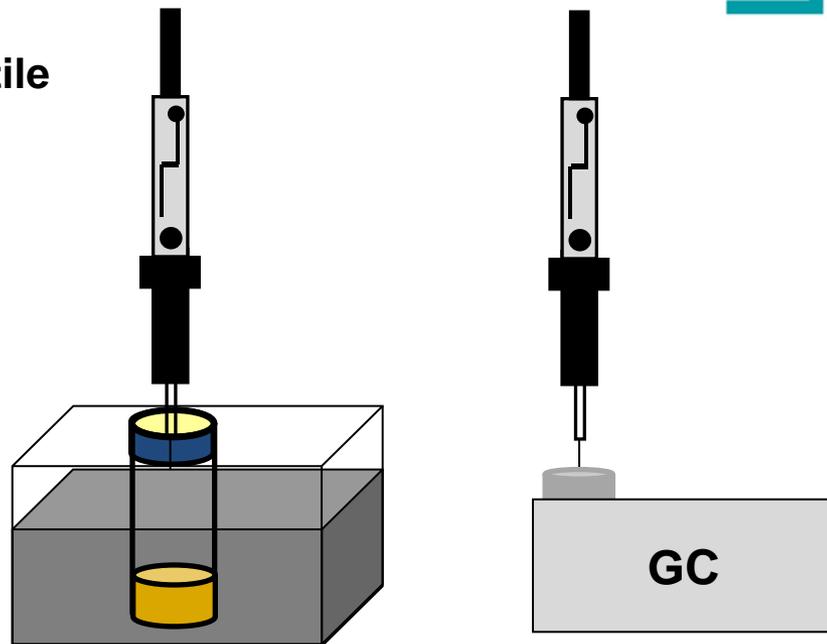
- *Sampling:* Headspace Solid Phase Micro-Extraction (HS-SPME)
- *Separation:* GC
- *Detection:* Time-of-Flight Mass Spectrometry (TOFMS)

[1] Marques. ASBC Presentation A30. Chicago, IL. June 2014.

Method Details

HS-SPME concentrates volatile and semi-volatile compounds in the headspace above a sample onto a fiber

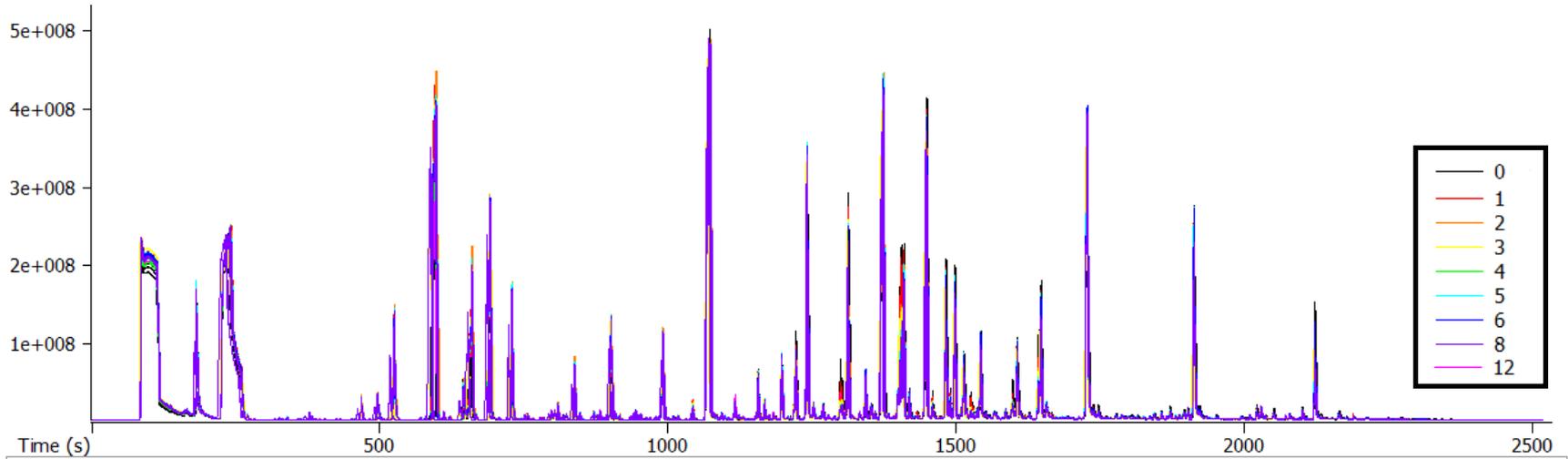
- 1) *Incubate 5 mL beer for 10 min at 60°C to drive compounds into the gas phase*
- 2) *Expose tri-phase SPME fiber (DVB/CAR/PDMS) to headspace for 20 min to collect volatile analytes*
- 3) *Expose fiber to GC inlet at 250°C for 3 min to desorb and inject analytes for analysis*



GC	Agilent 7890 with Gerstel Autosampler
Column	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Carrier Gas	He @ 1 ml/min
Oven Program	4 min at 35°C, ramp 5°C/min to 180°C, ramp 10°C/min to 220°C hold 5 min
MS	LECO Pegasus® HT
Ion Source Temp	250 °C
Mass Range	35-500 m/z
Acquisition Rate	20 spectra/s



GC-TOFMS Data

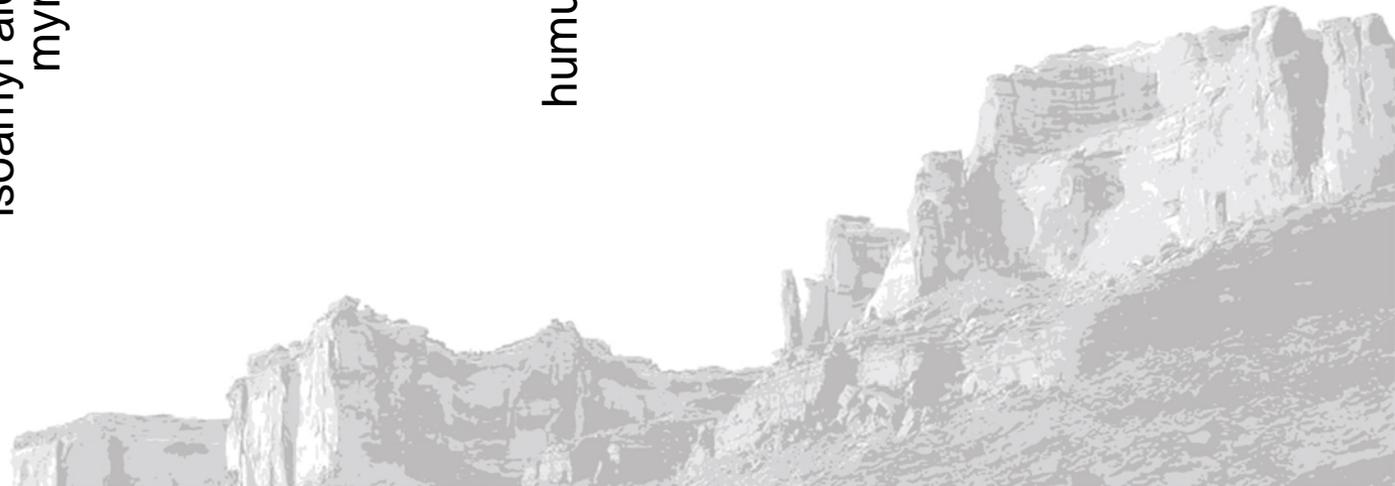
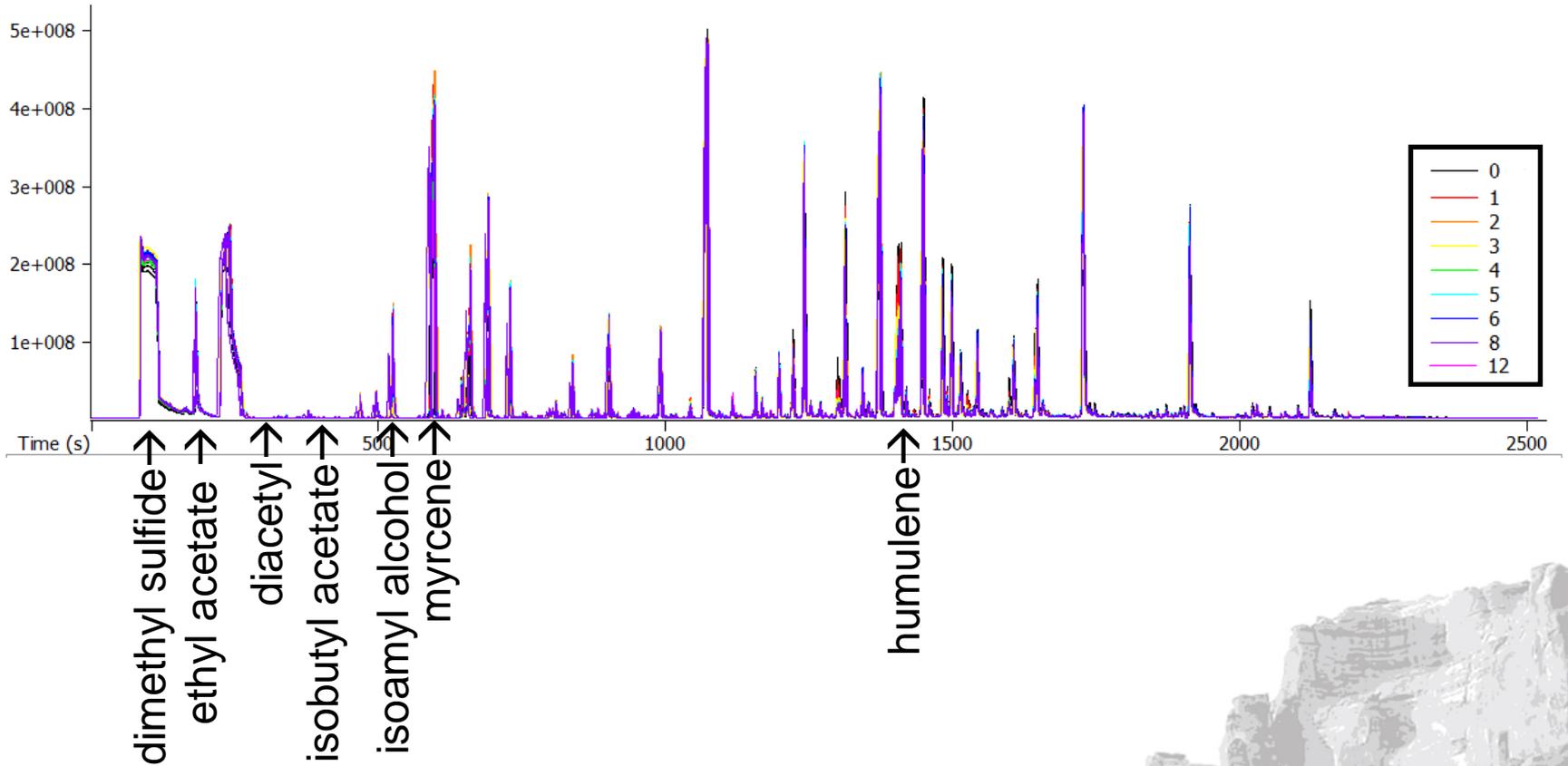


**Overlay of replicate injections of all time points:
0, 1, 2, 3, 4, 5, 6, 8, and 12**

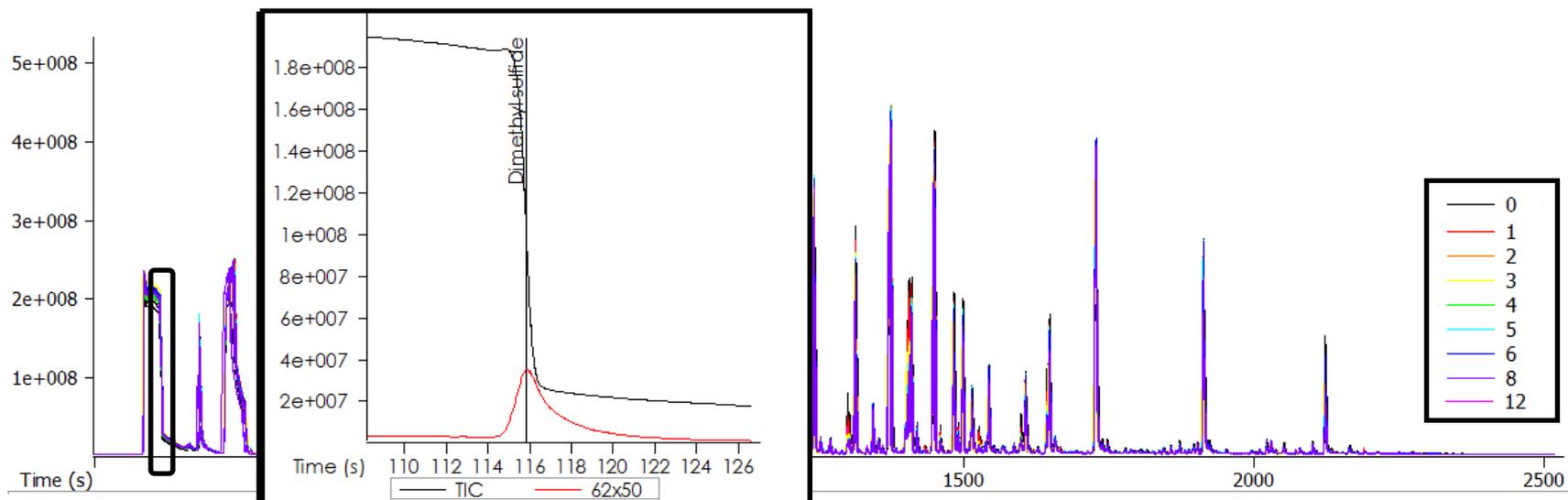
Review data for analytes of known interest



Target Review



Dimethyl Sulfide – sulfurous/onion/corn

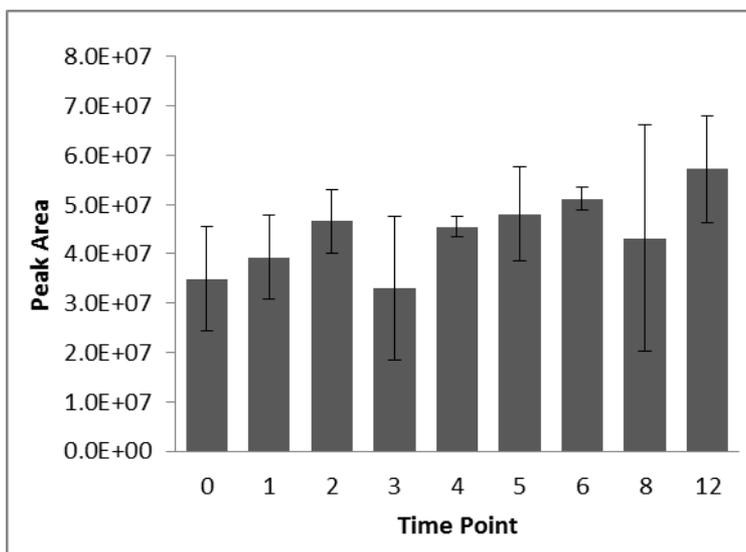
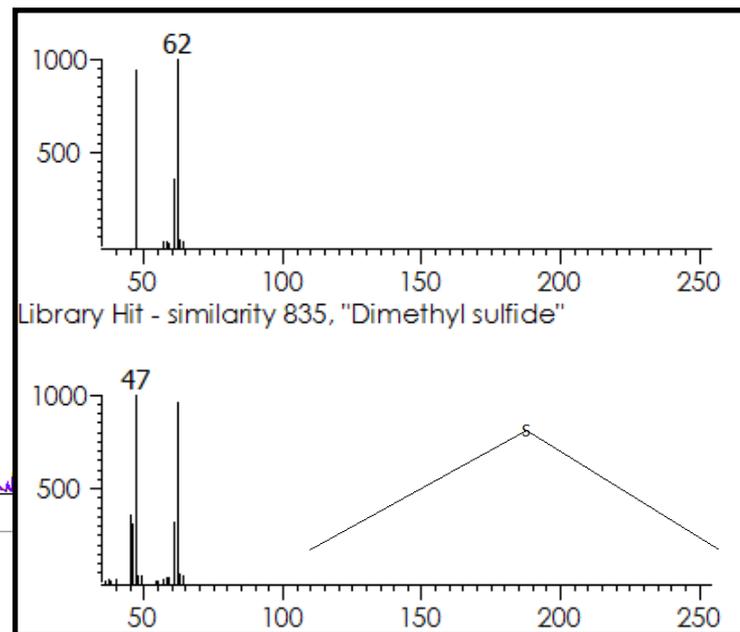
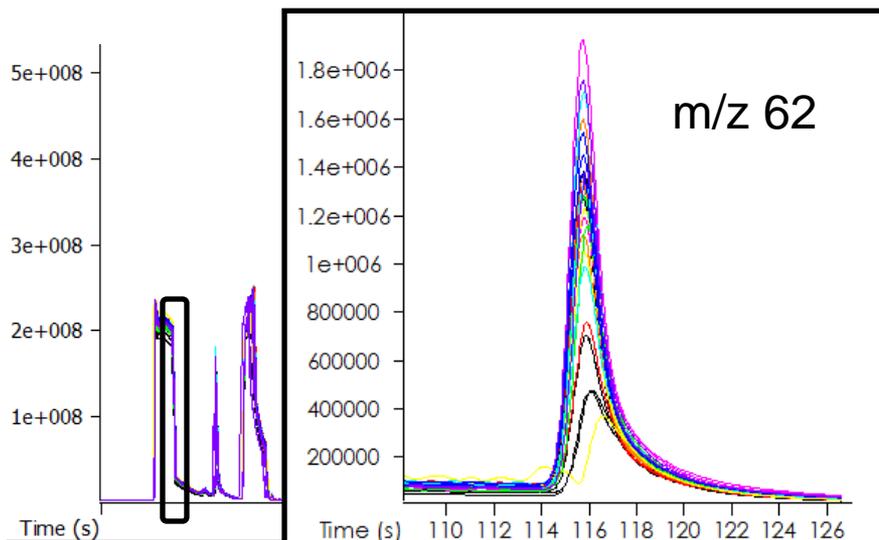


Peak finding isolates analyte
from background

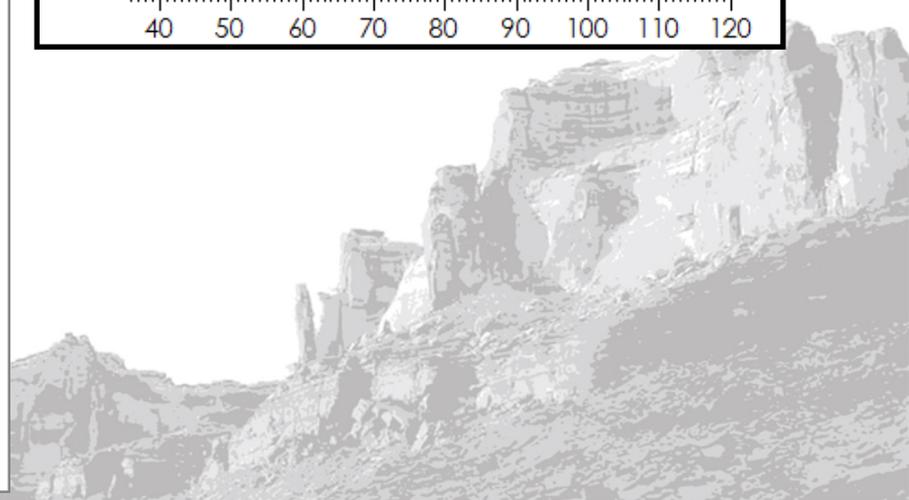
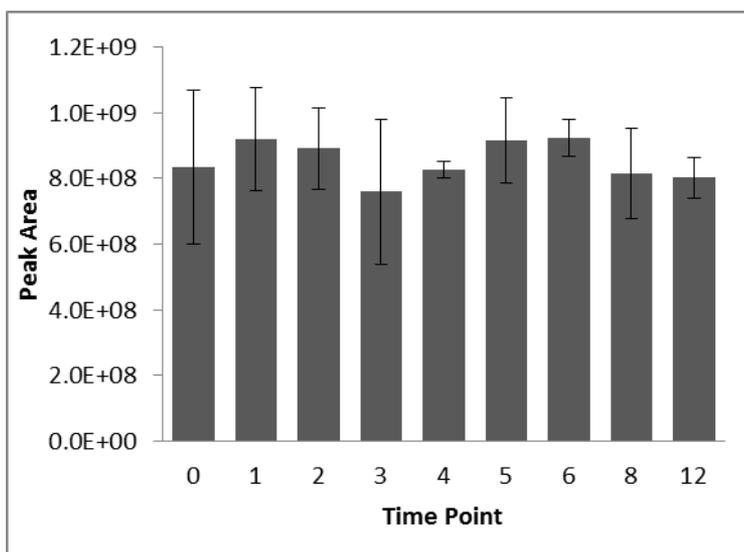
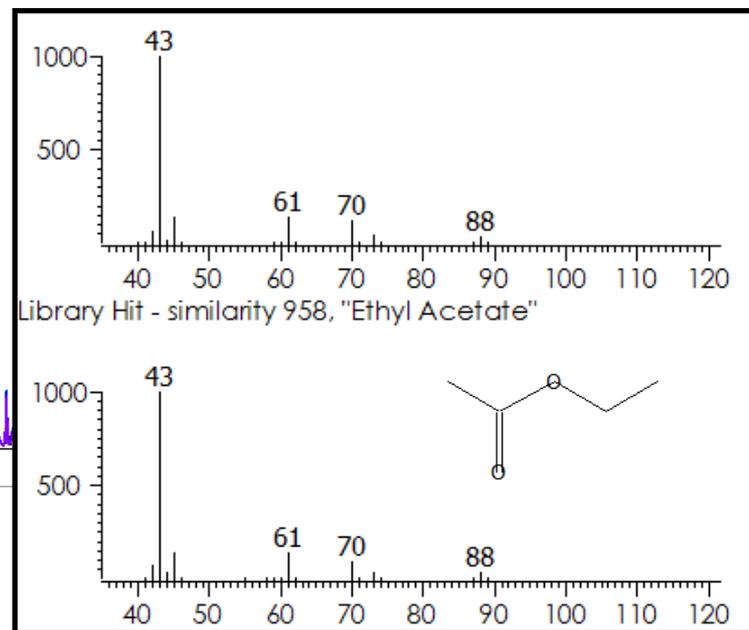
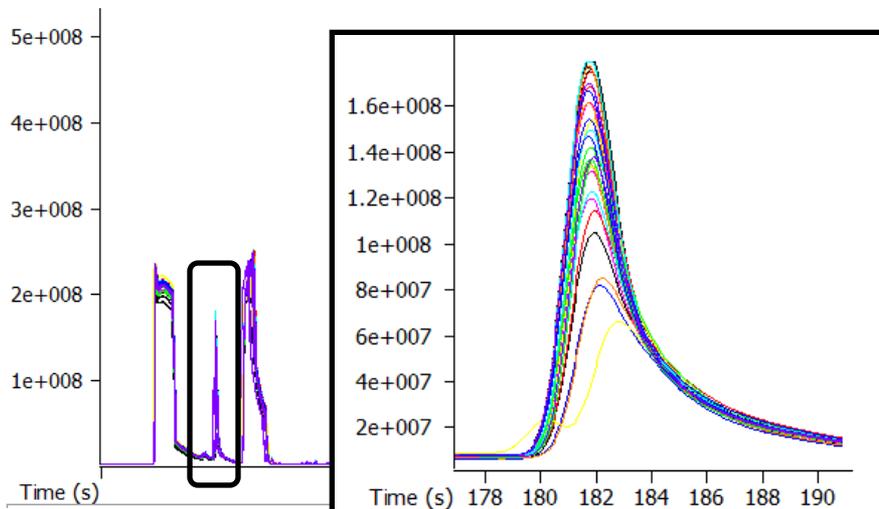




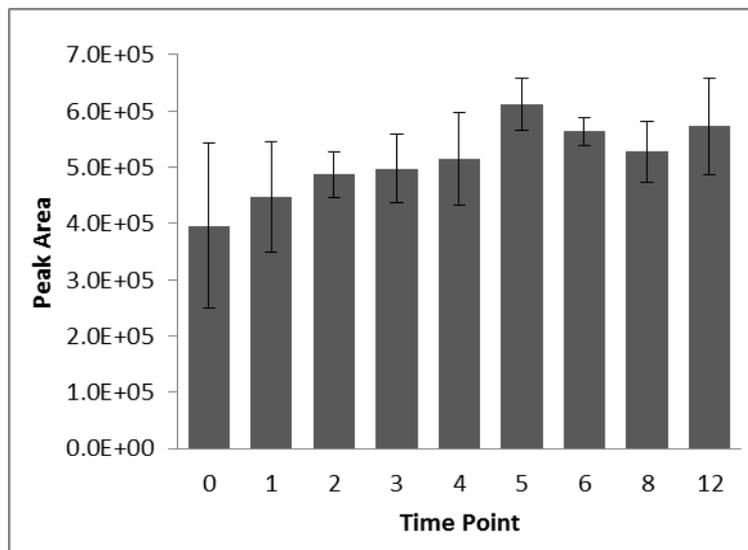
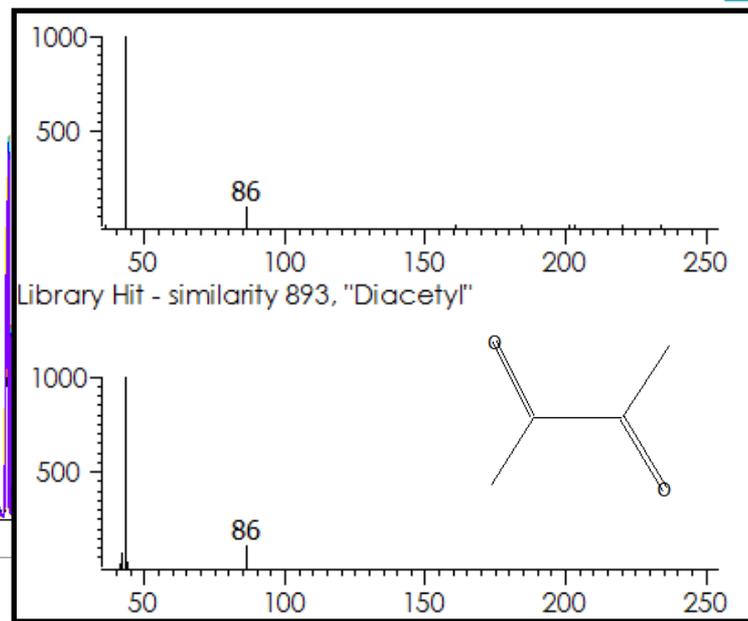
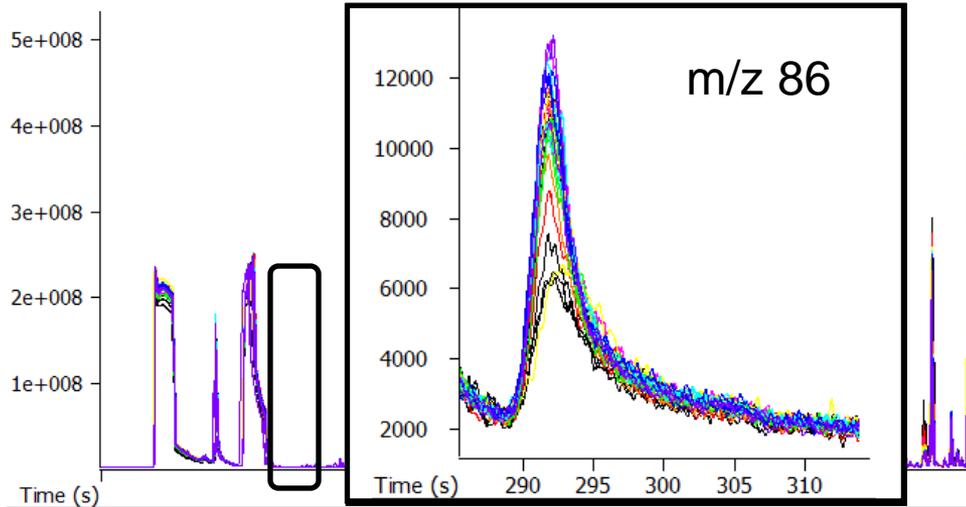
Dimethyl Sulfide – sulfurous/onion/corn



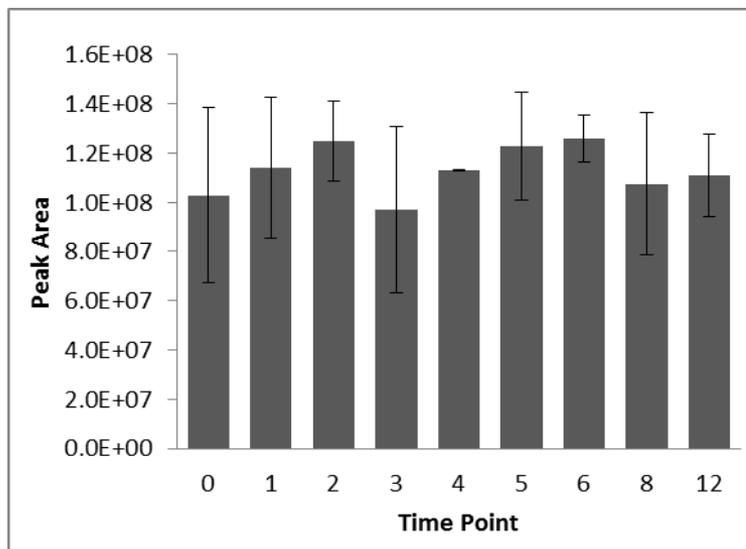
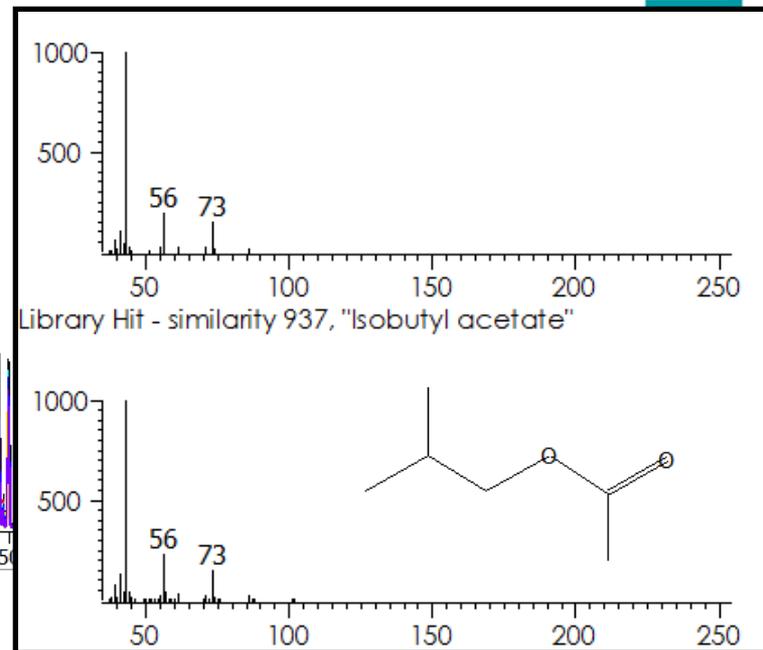
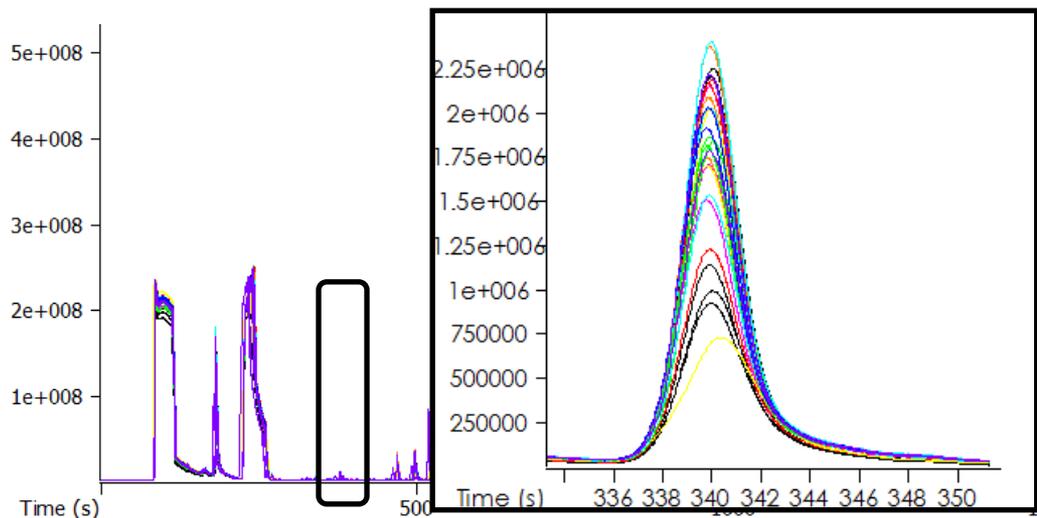
Ethyl Acetate – ethereal/fruity ester



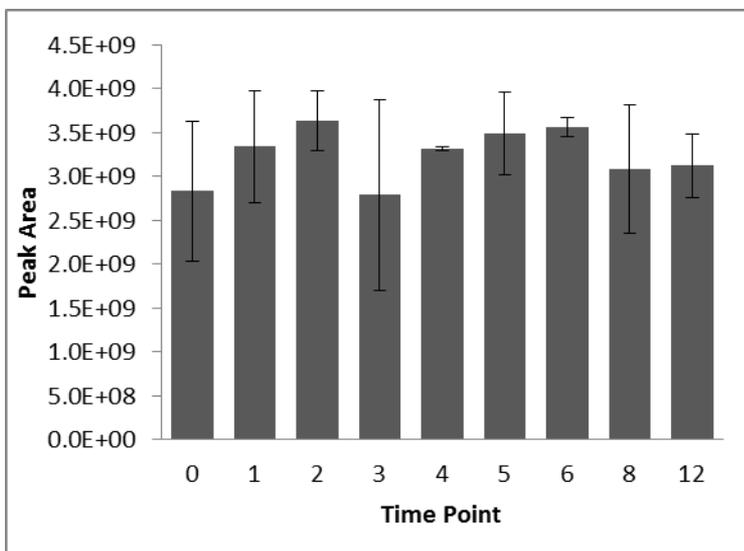
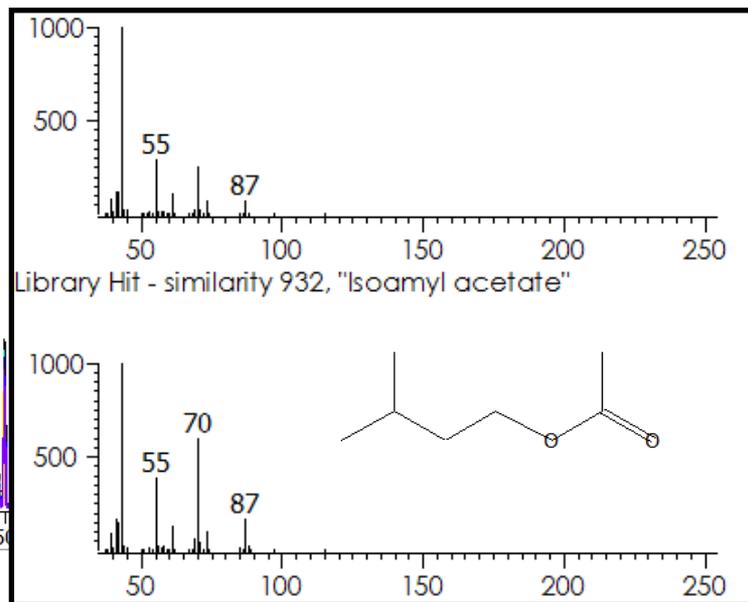
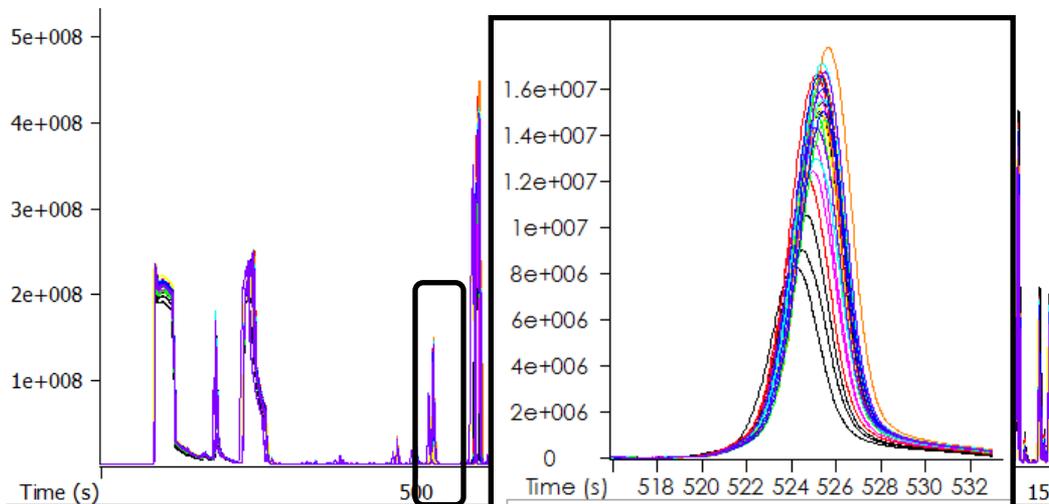
Diacetyl – buttery/pungent



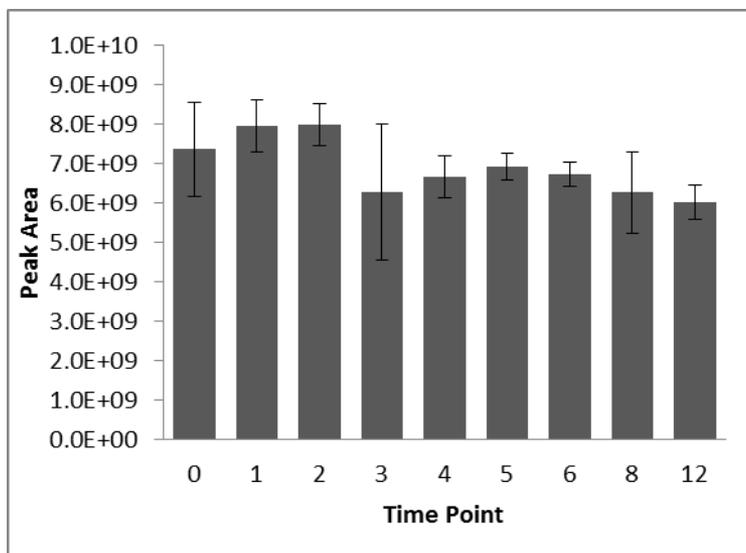
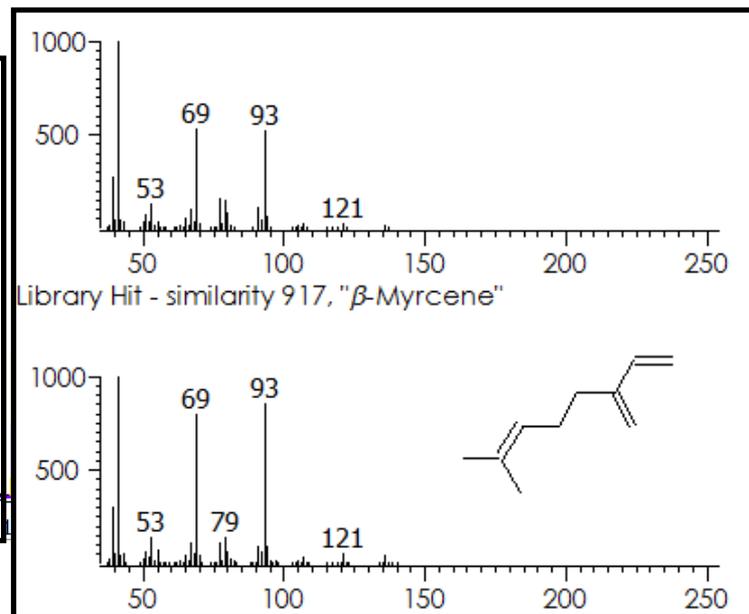
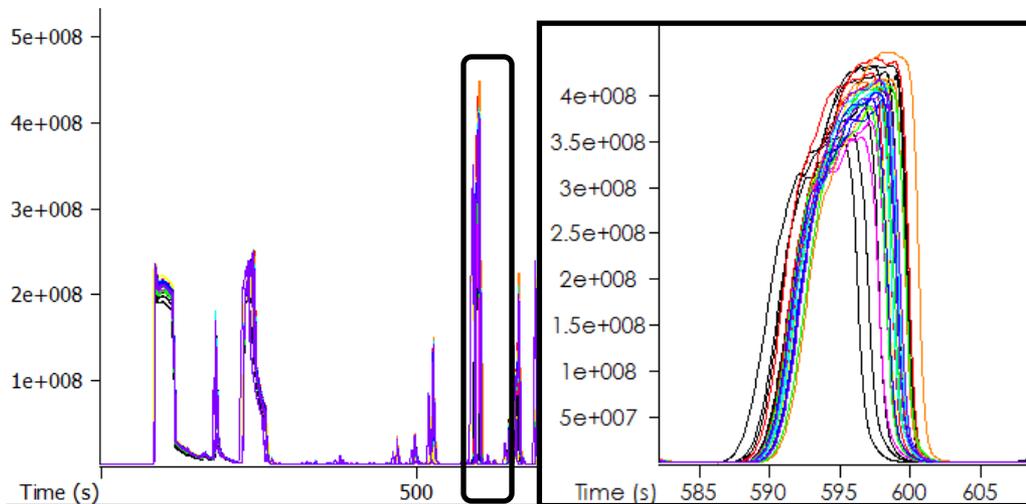
Isobutyl acetate – fruity ester



Isoamyl Acetate – banana ester

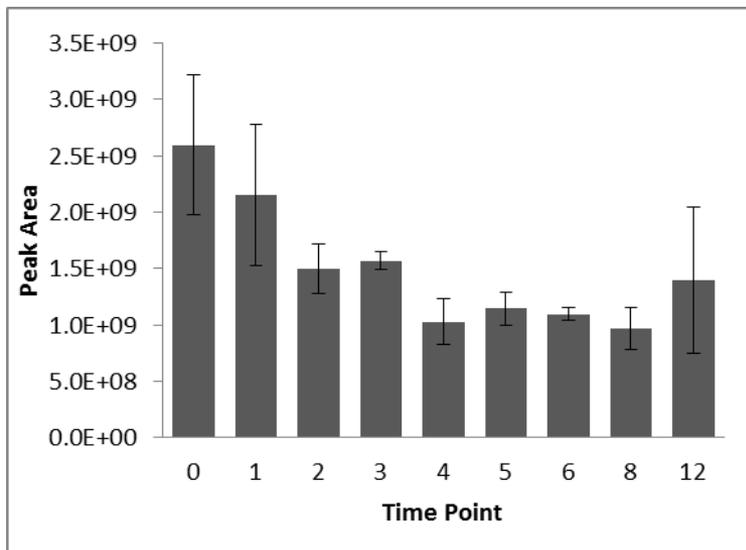
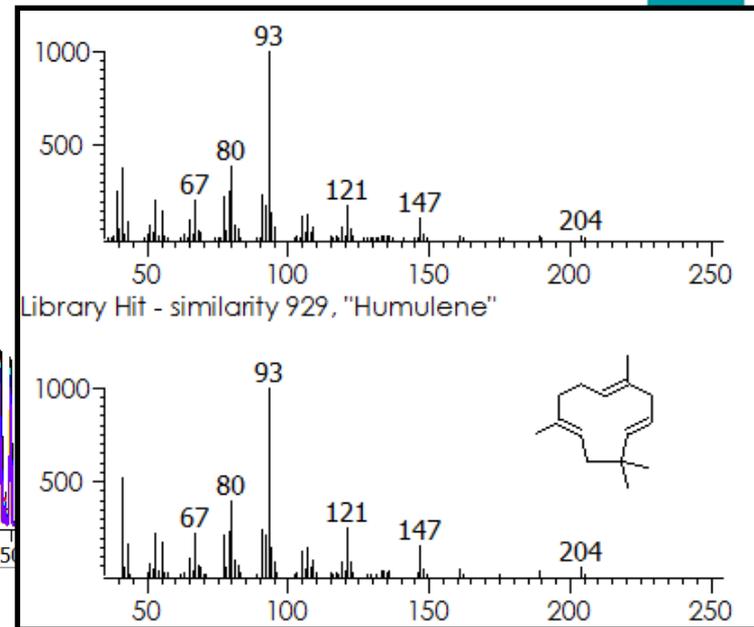
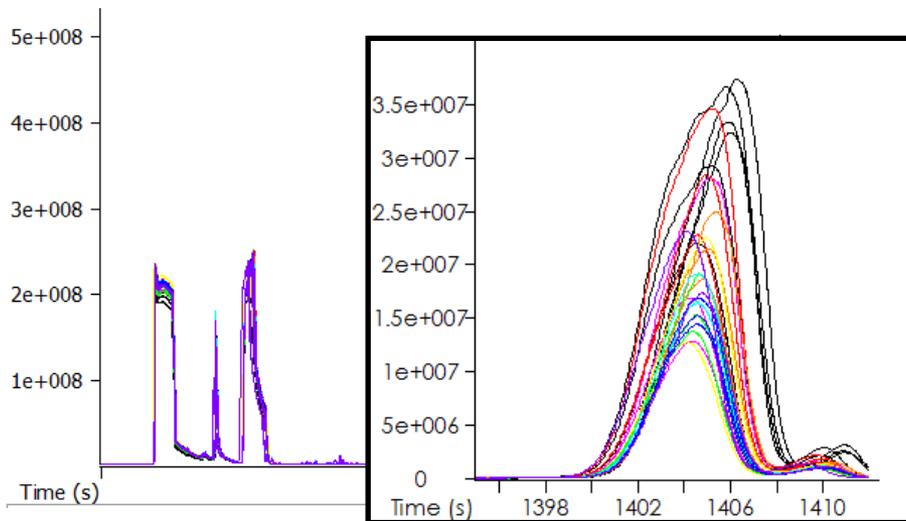


Myrcene – peppery/spicy terpene



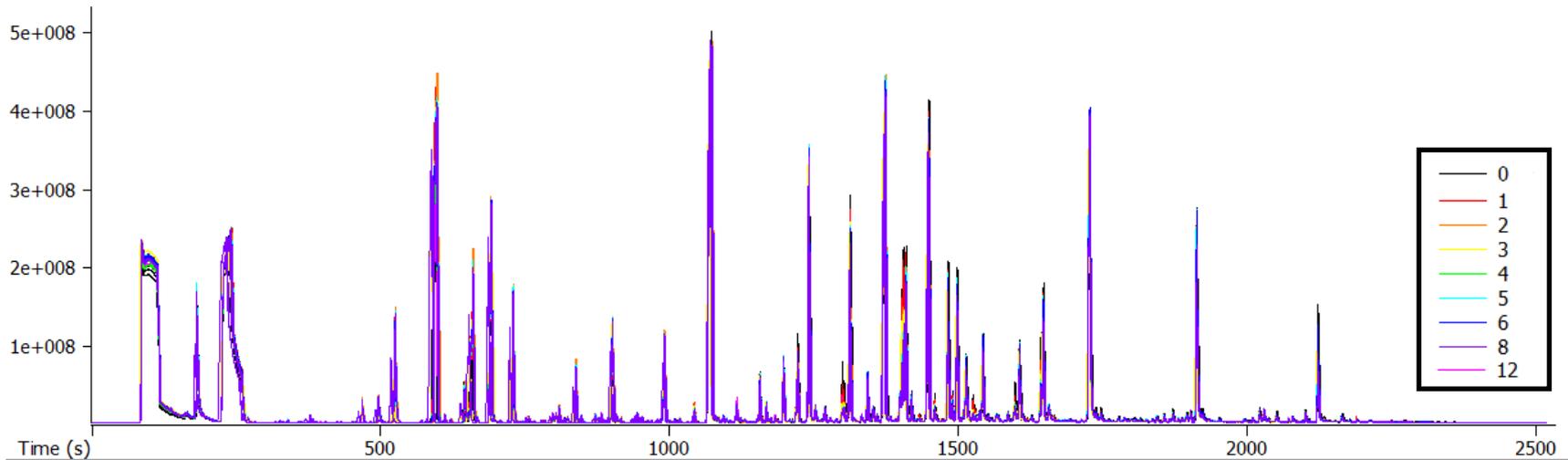


humulene – woody terpene





Non-targeted Analysis

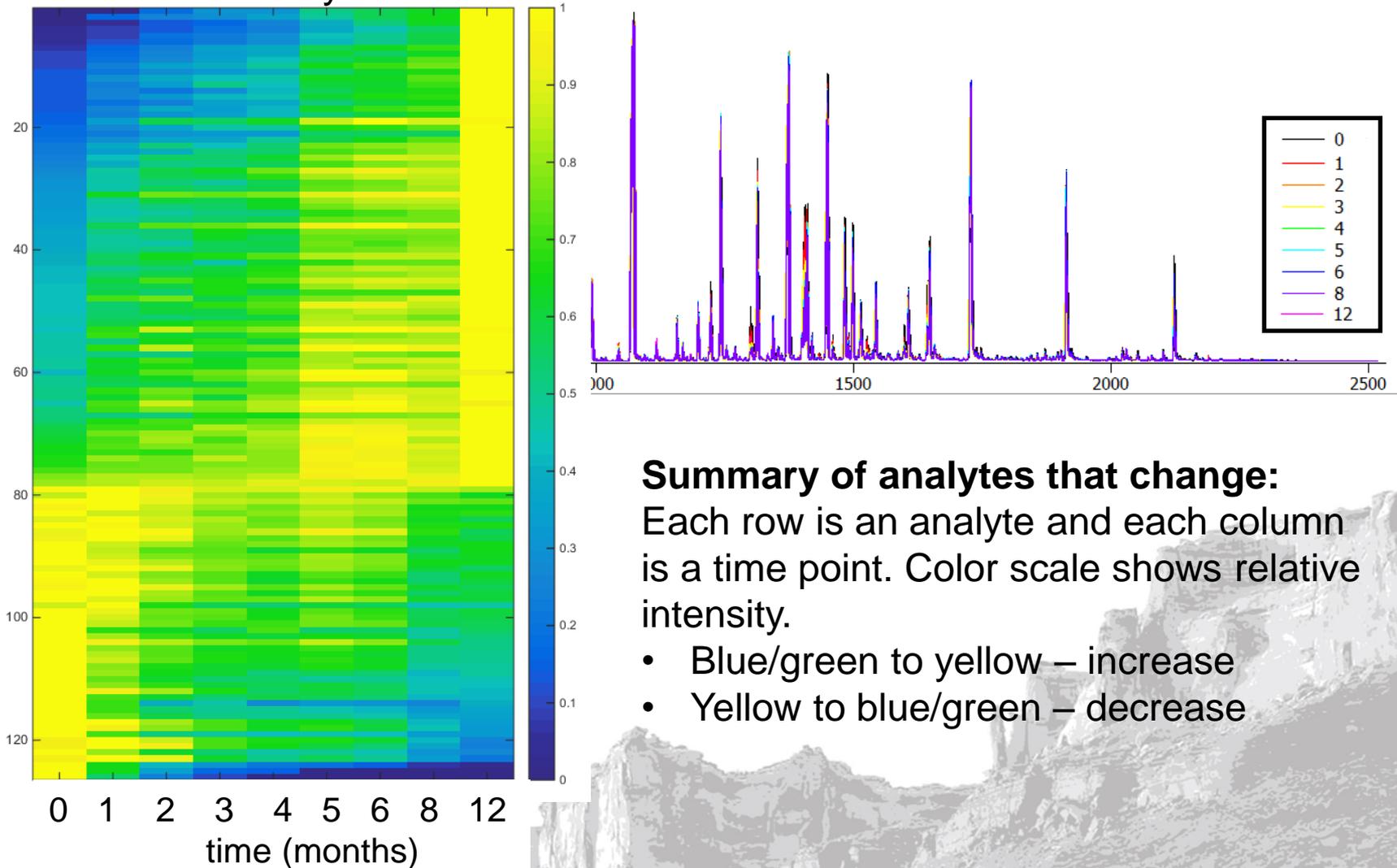


Discovery with Non-targeted Analysis

- 1) Compile peak area information for >400 analytes
Esters, terpenes, alcohols, aldehydes/ketones, sulfur containing, heterocyclic
- 2) Perform t-test between data from time 0 and time 12
143 significantly differ at 90% confidence
- 3) Review regression statistics for time course
127 exhibit a time course trend based on R^2
- 4) Group analytes that trend up or down based on slopes
78 increase and 49 decrease

Time Course Trends

127 Analytes



Summary of analytes that change:

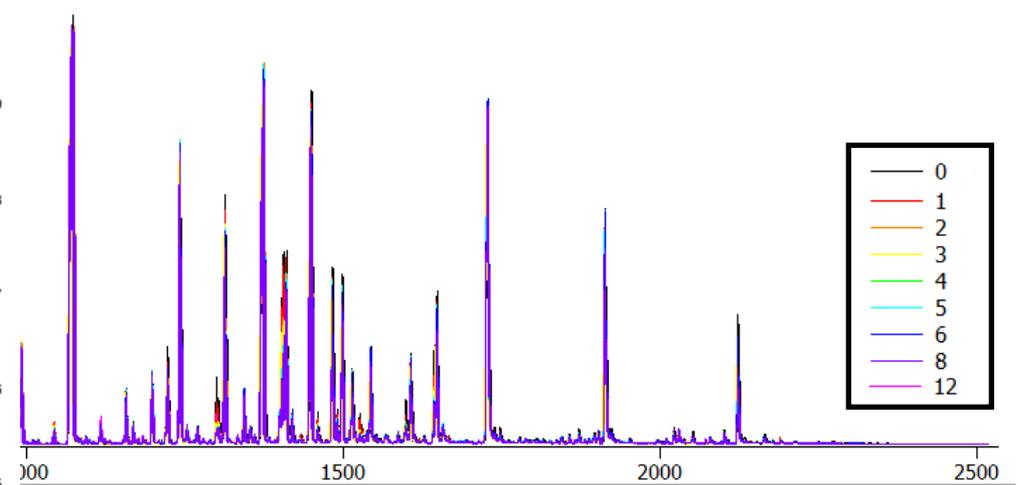
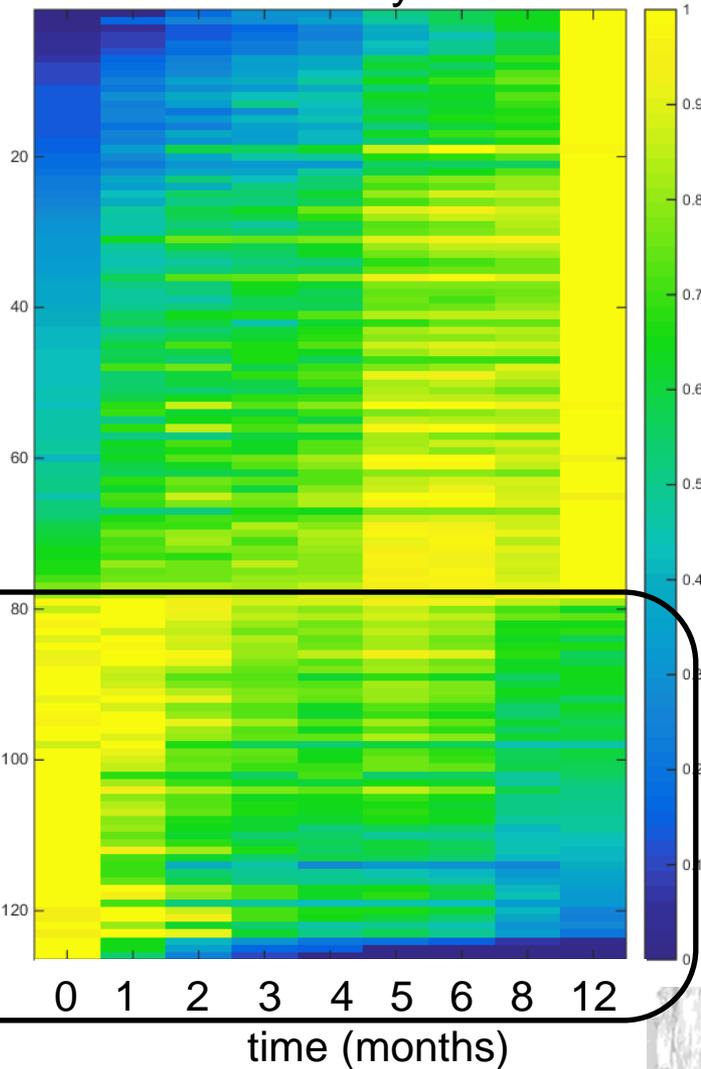
Each row is an analyte and each column is a time point. Color scale shows relative intensity.

- Blue/green to yellow – increase
- Yellow to blue/green – decrease

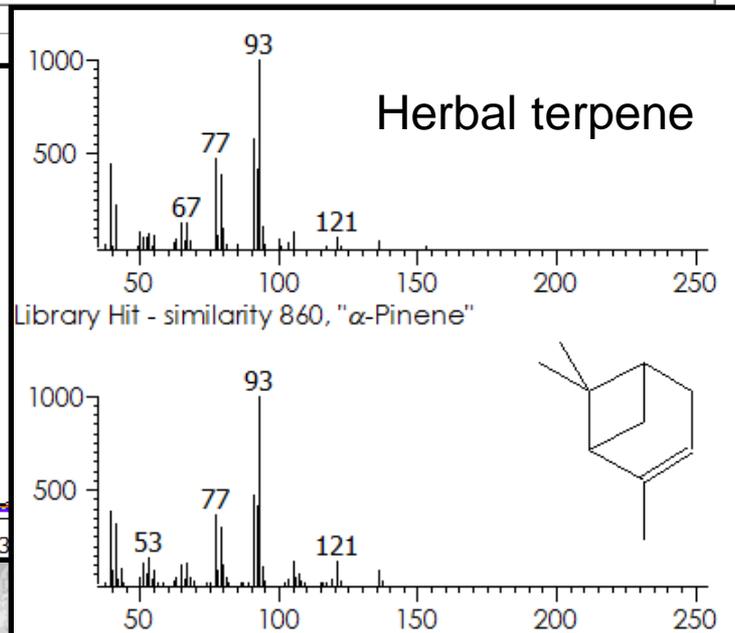
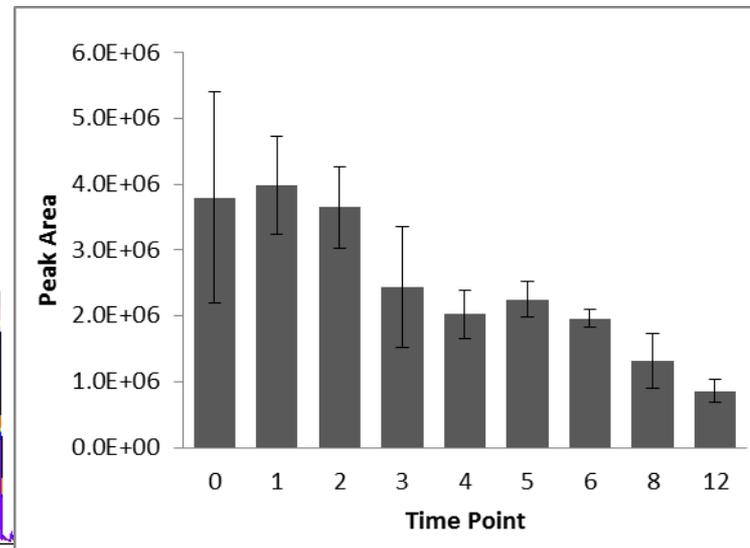
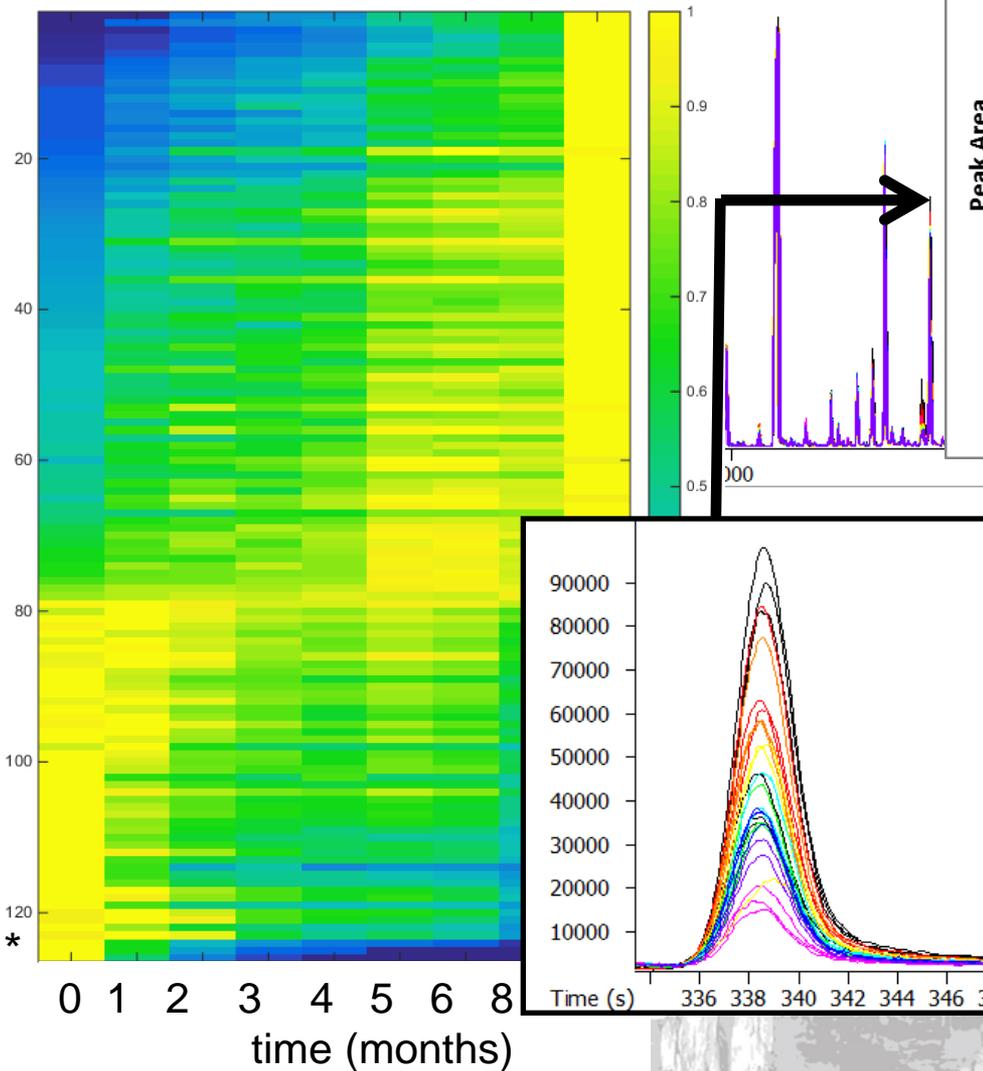


Analytes that Decrease with Age

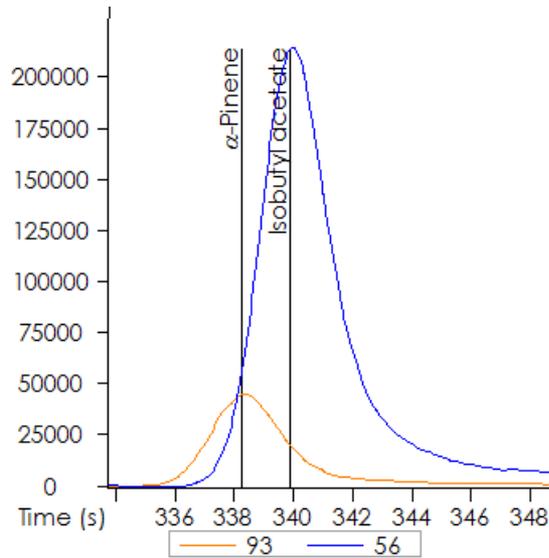
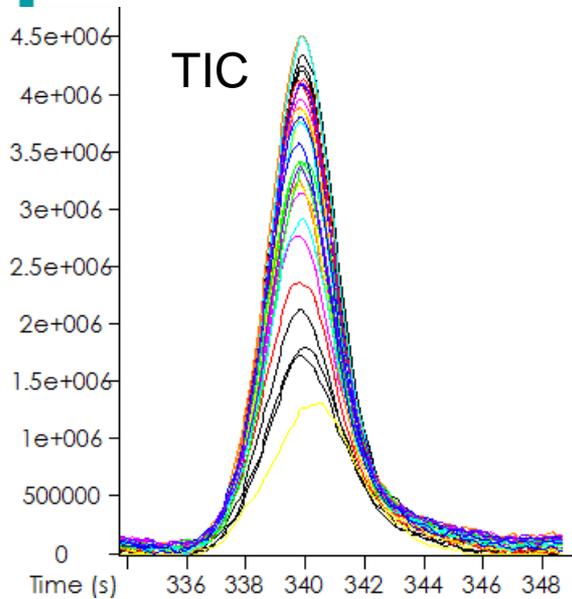
127 Analytes



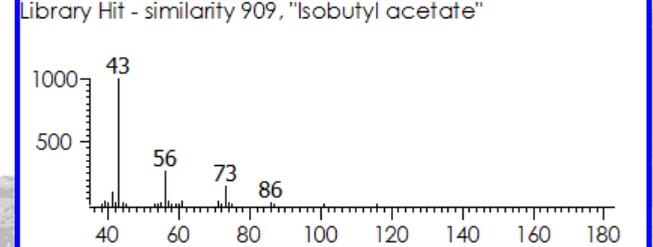
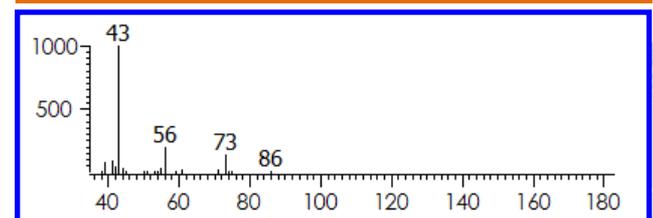
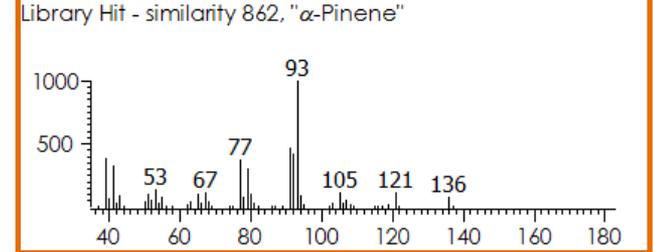
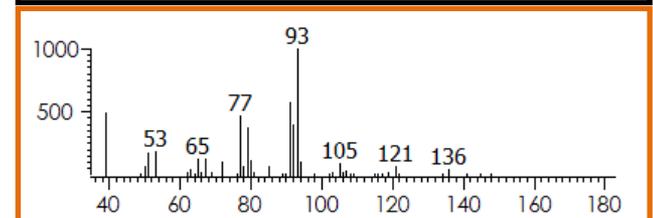
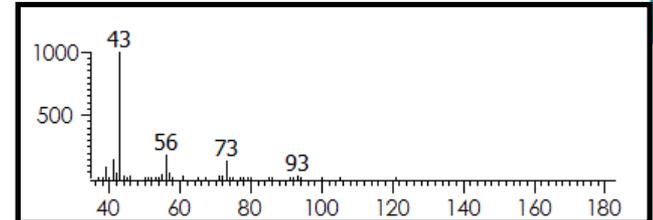
Analytes that Decrease with Age



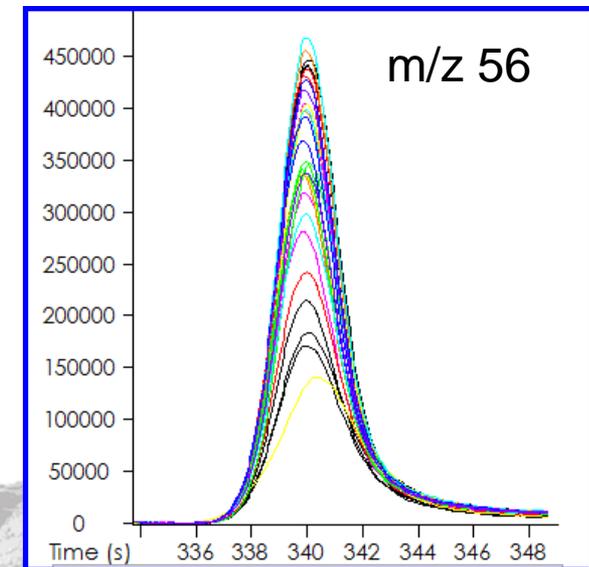
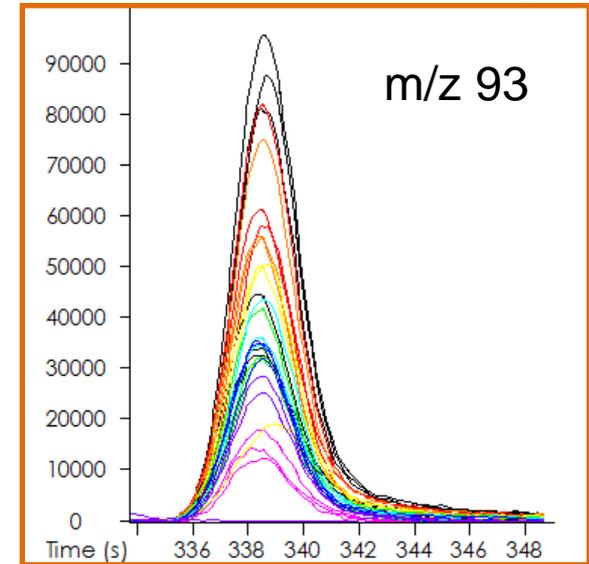
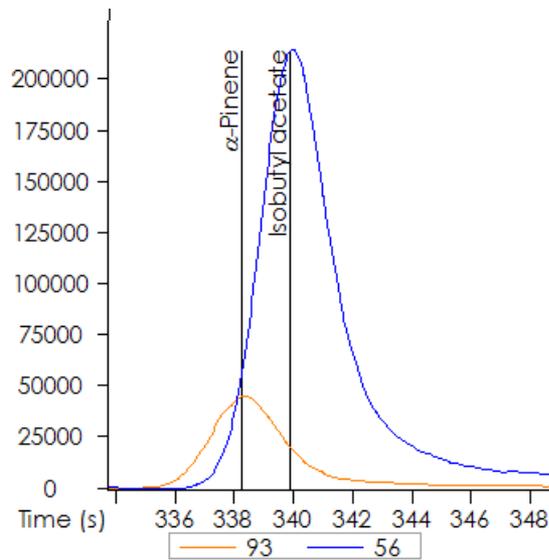
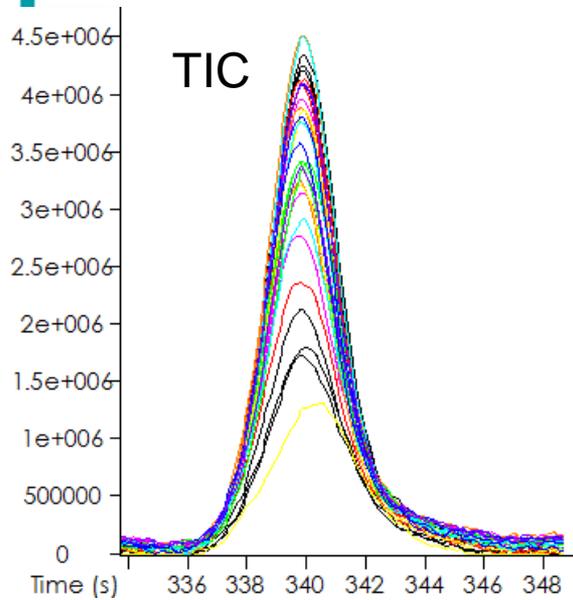
Benefit of Deconvolution



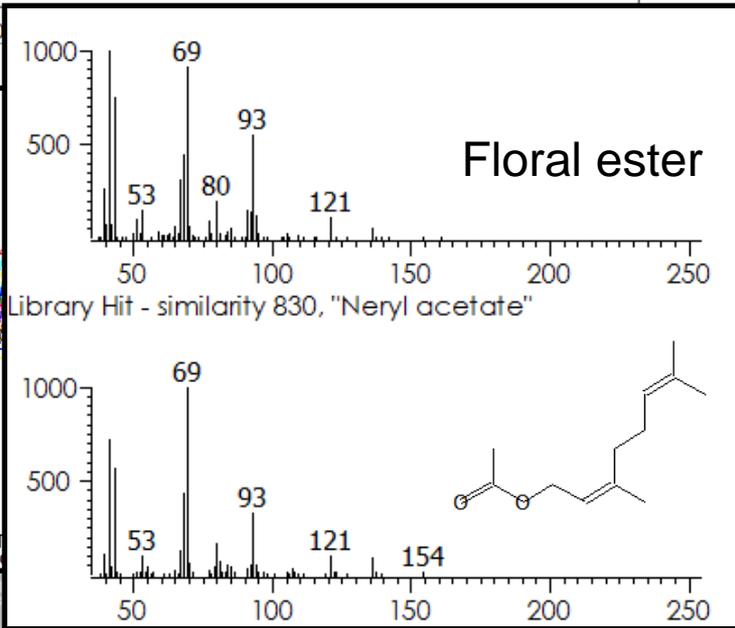
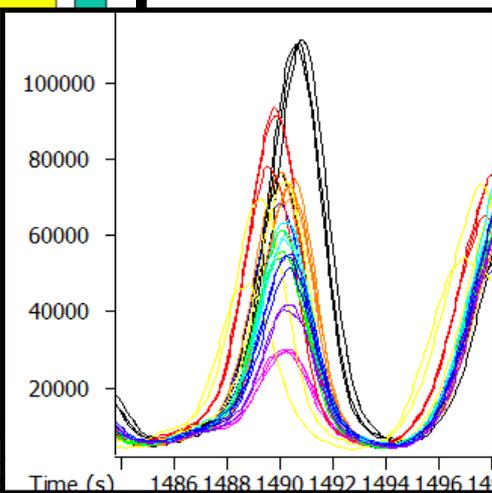
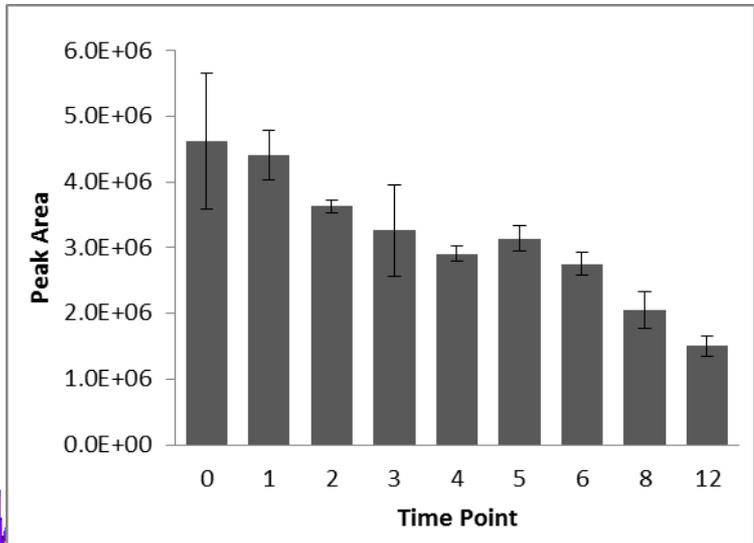
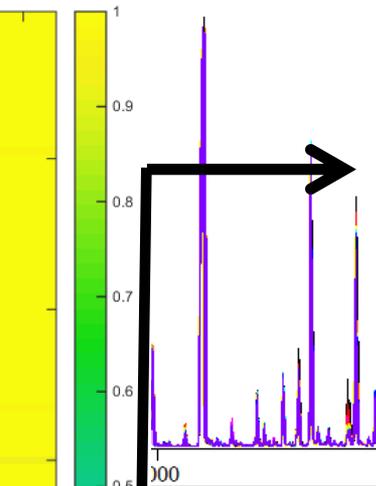
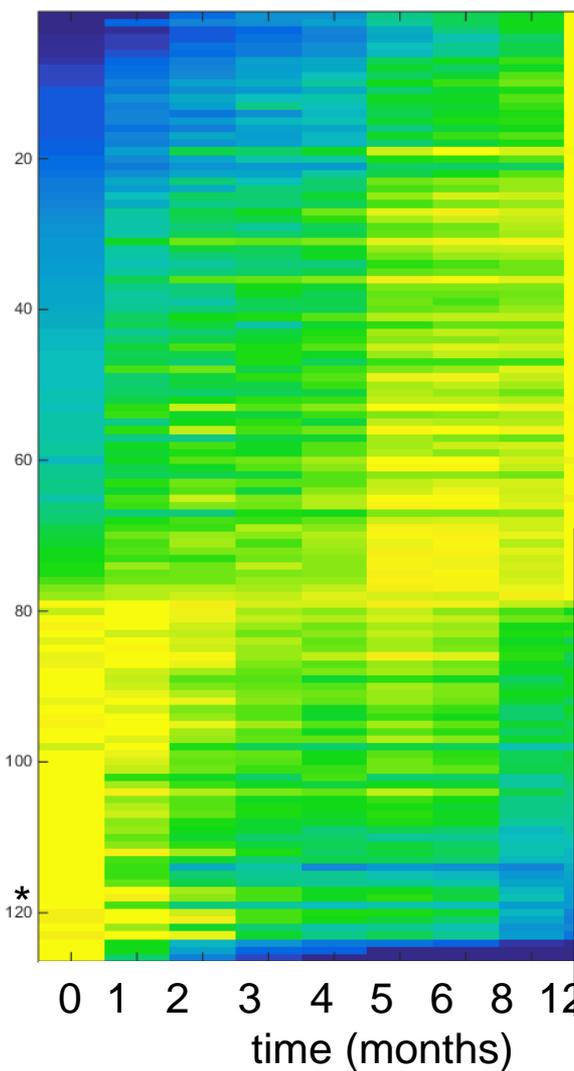
The nontarget analyte coelutes with the target, isobutyl acetate



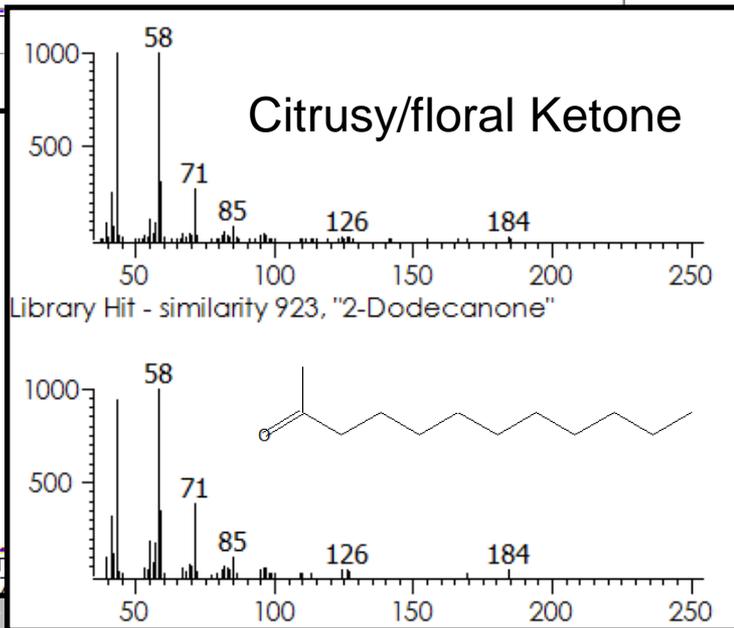
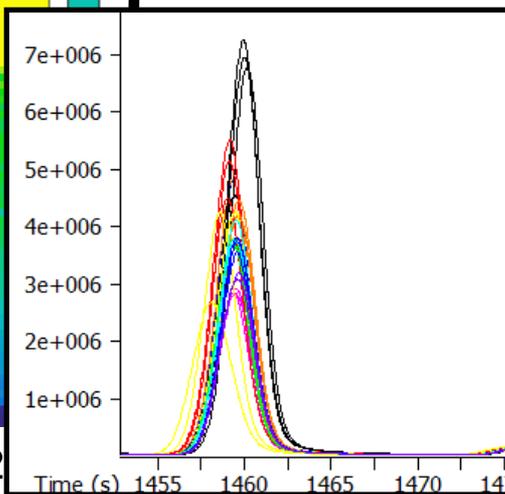
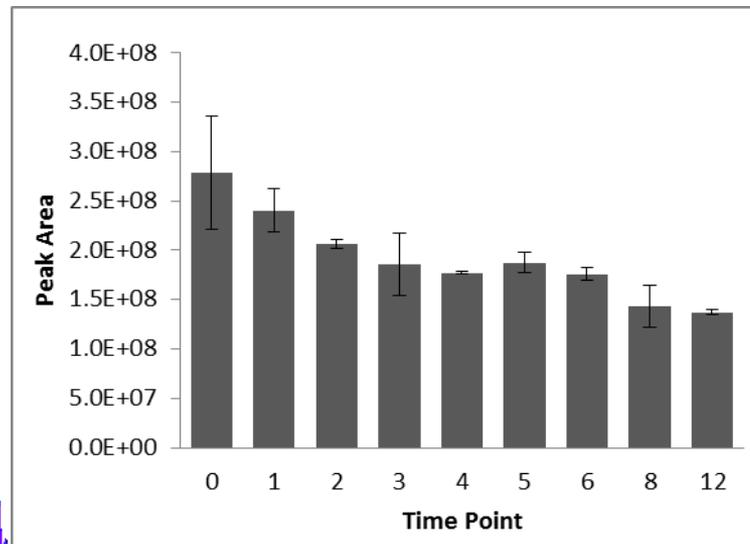
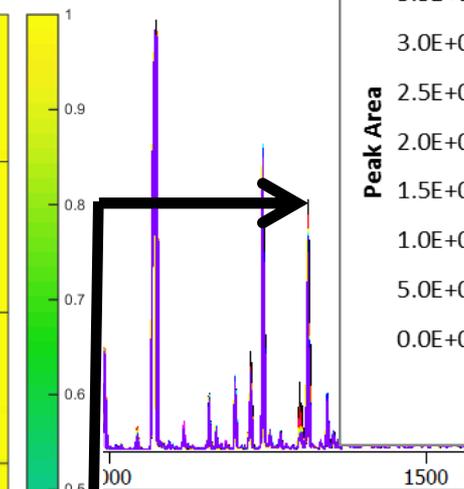
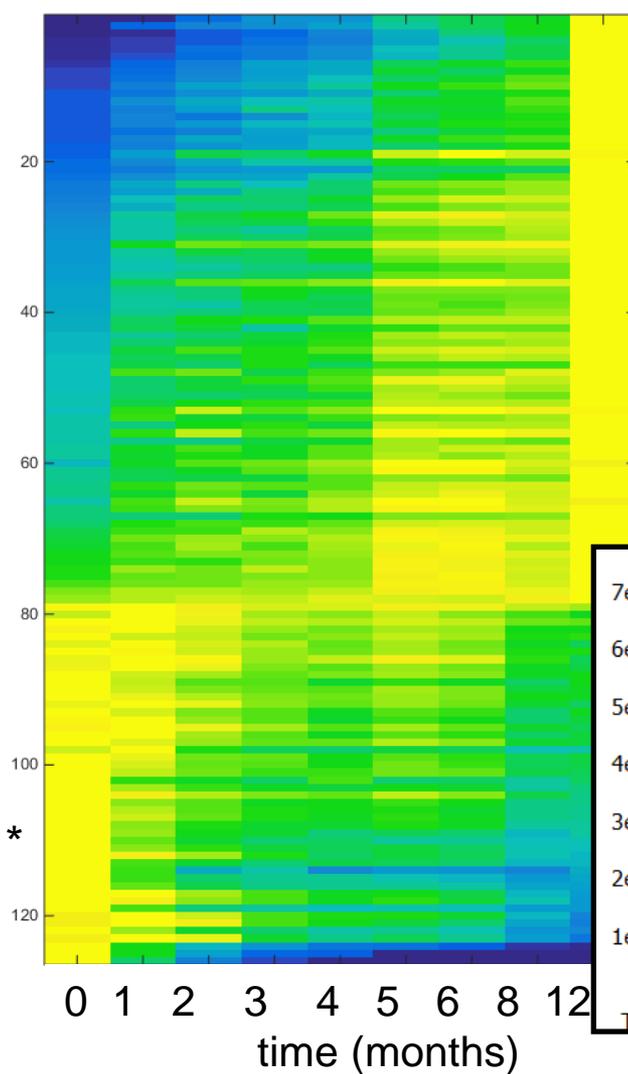
Benefit of Deconvolution



Analytes that Decrease with Age



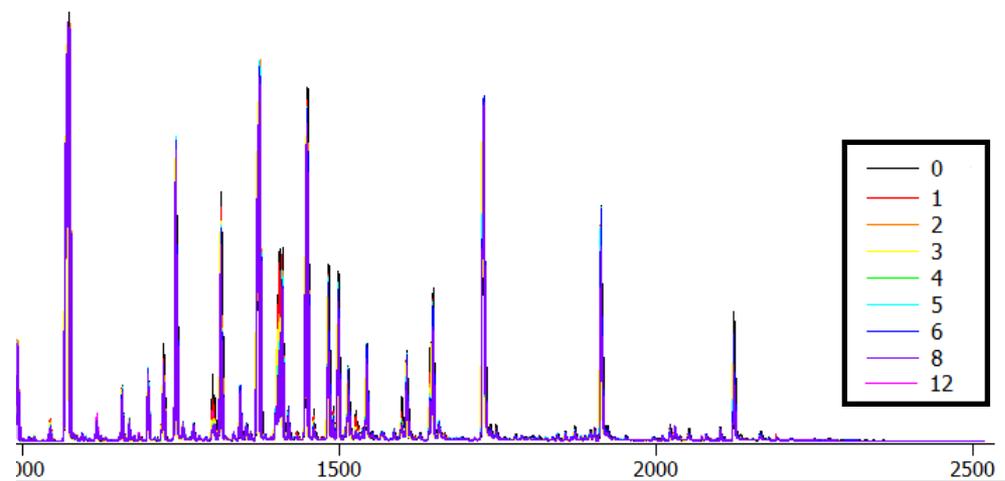
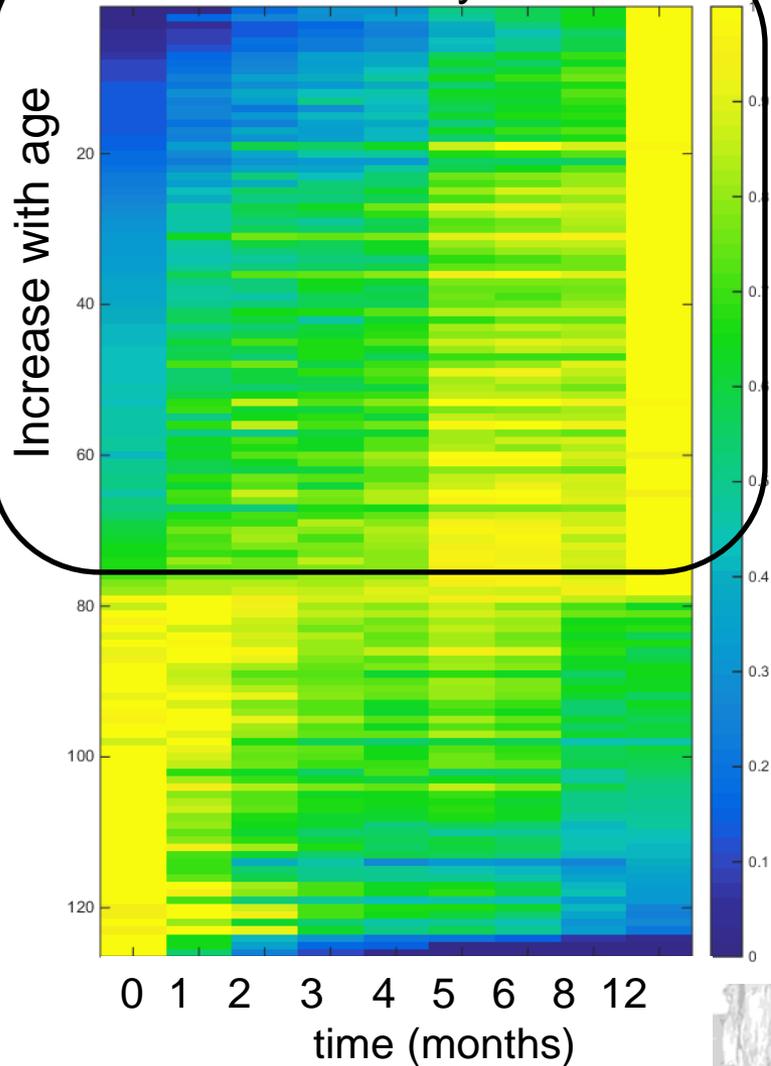
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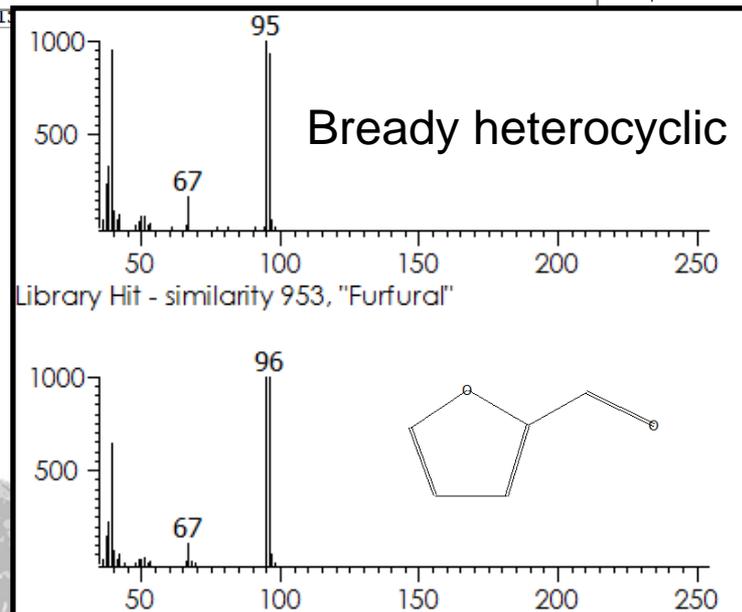
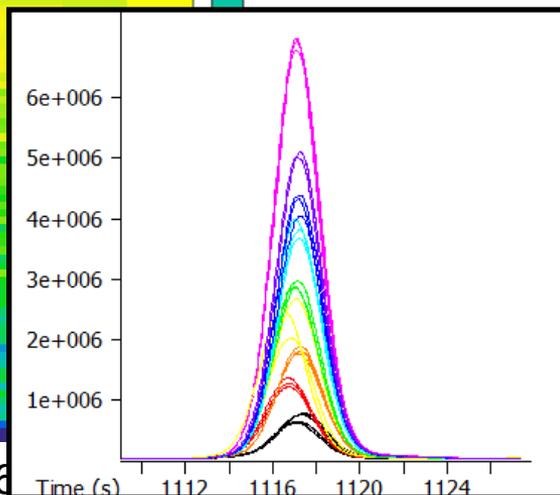
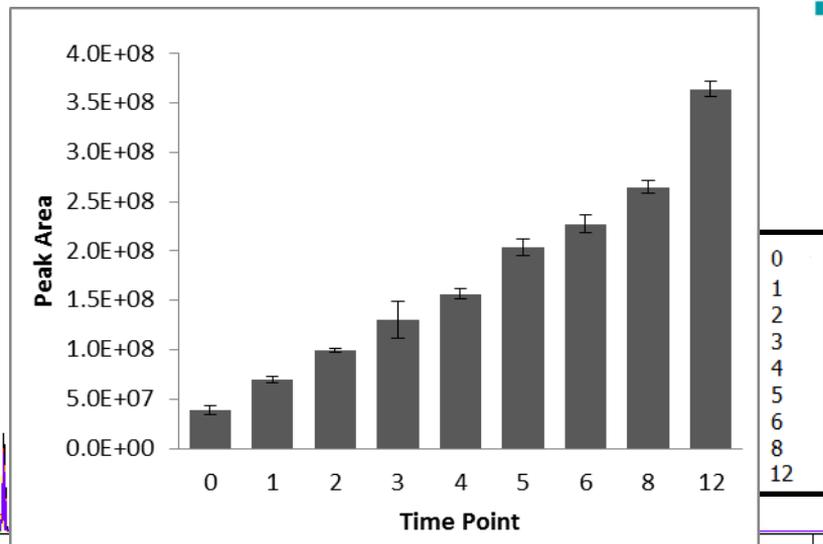
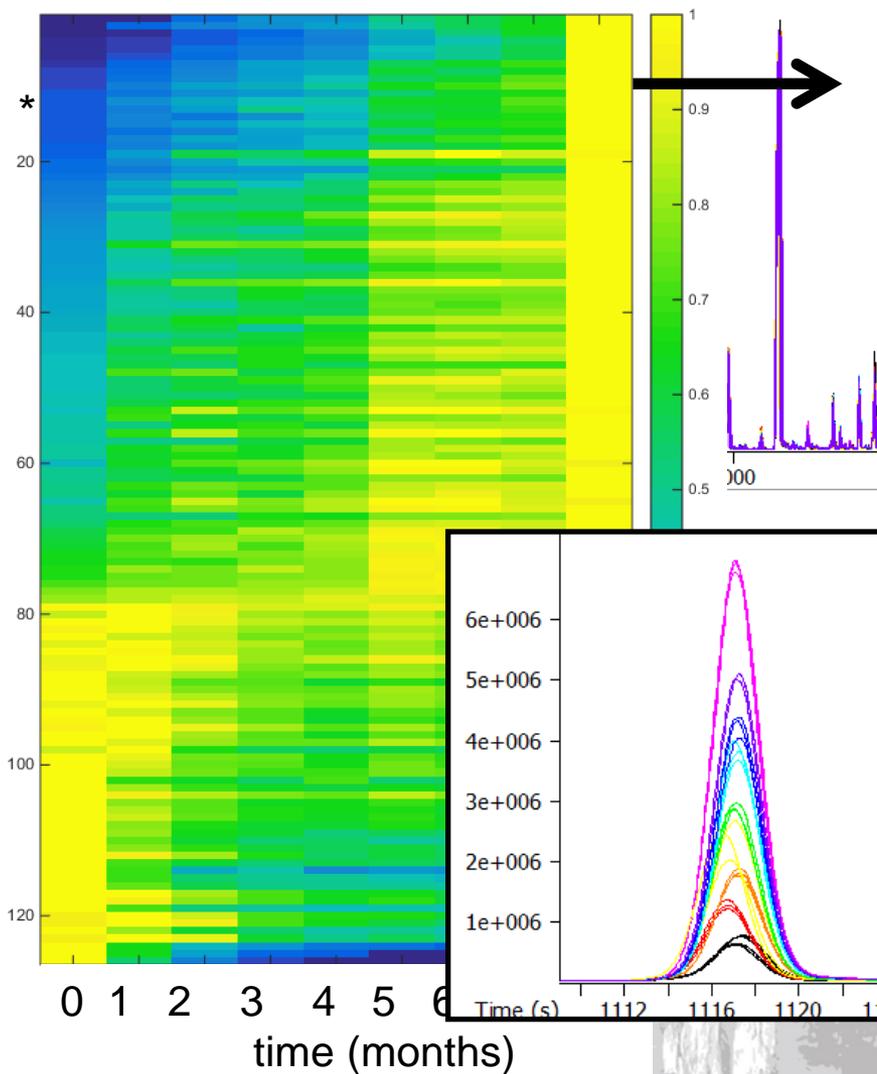


Analytes that Increase with Age

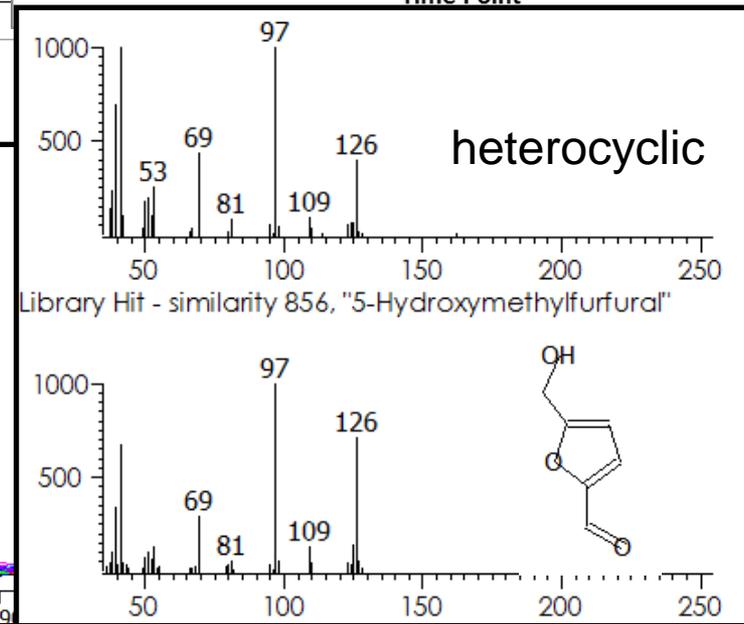
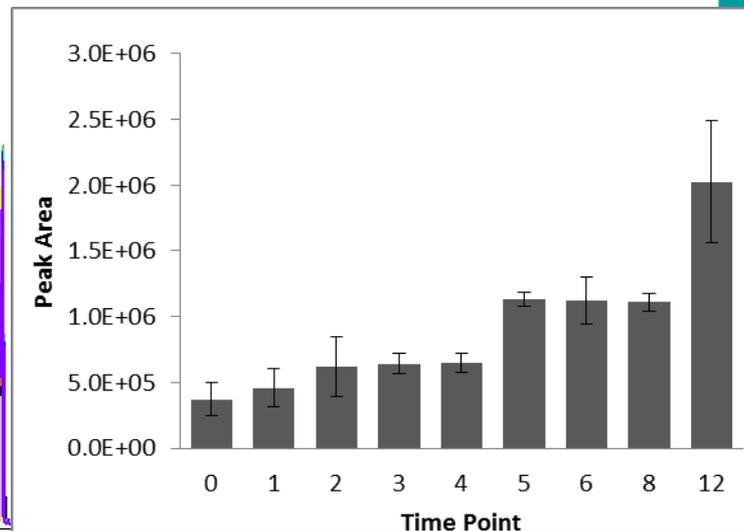
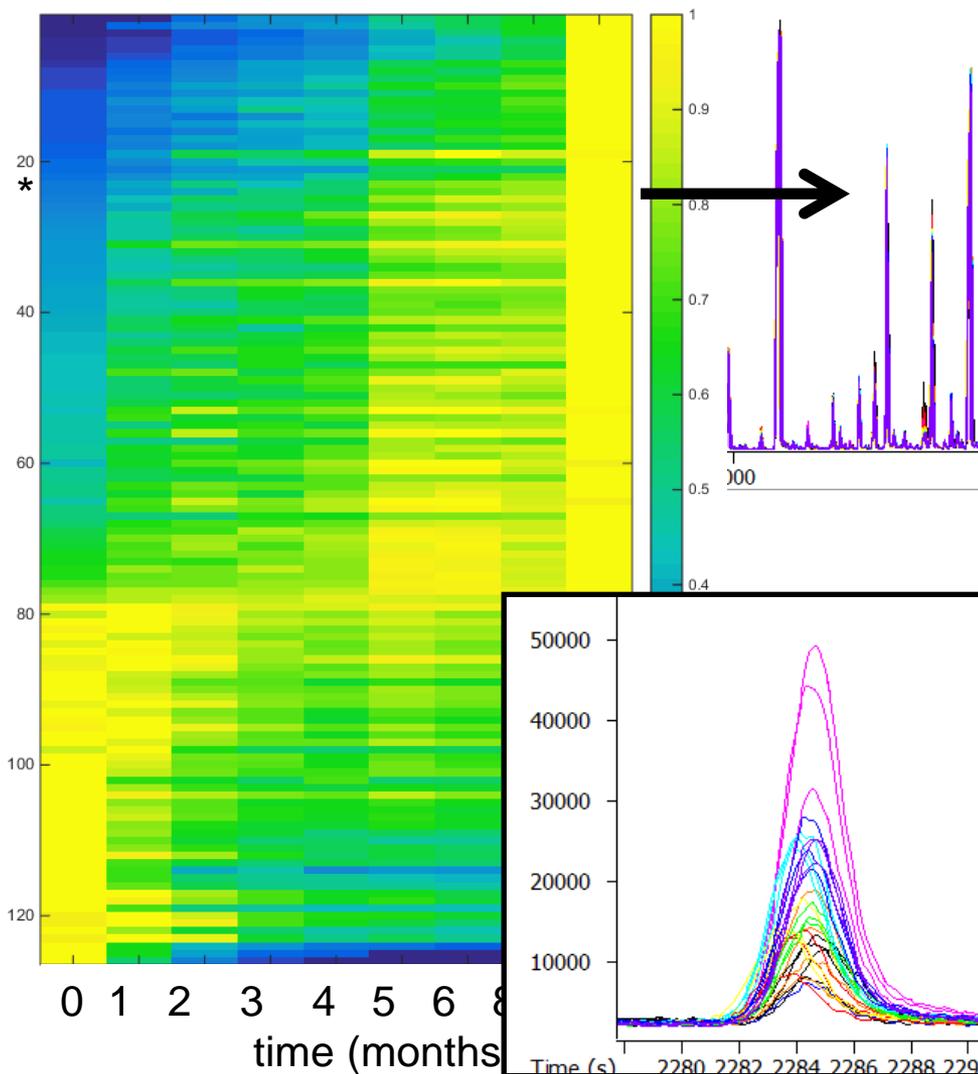
127 Analytes



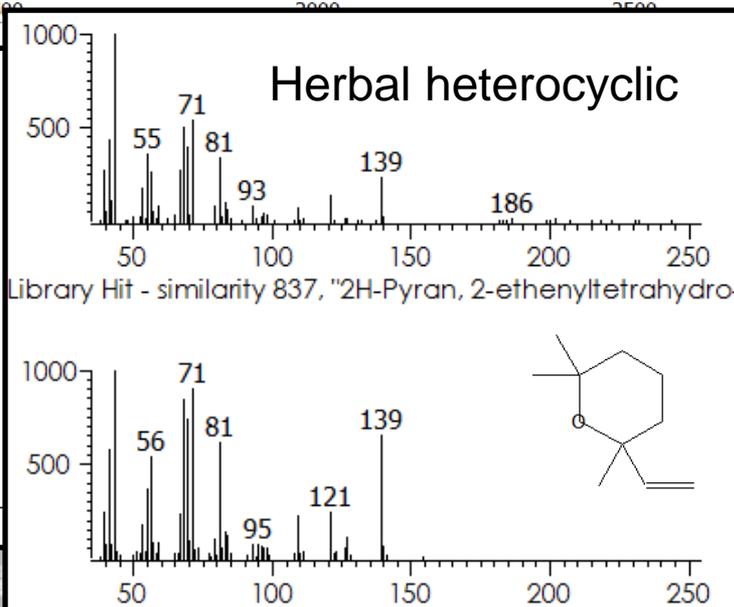
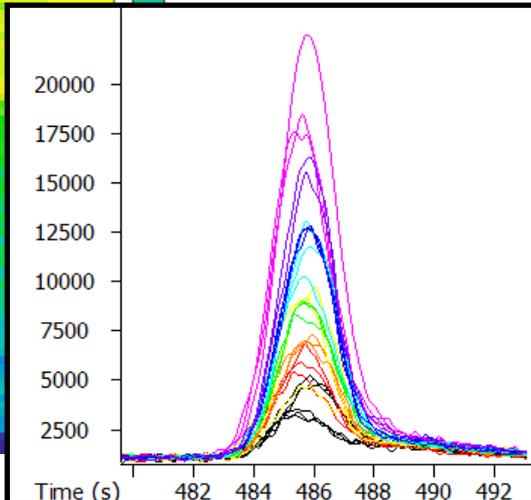
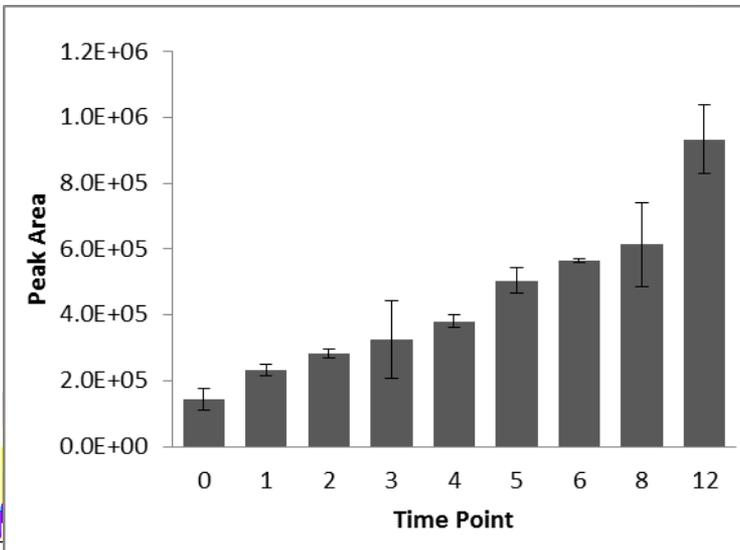
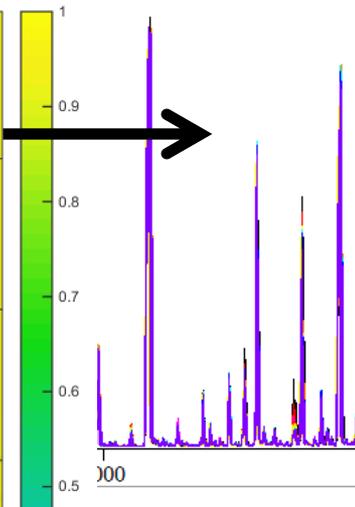
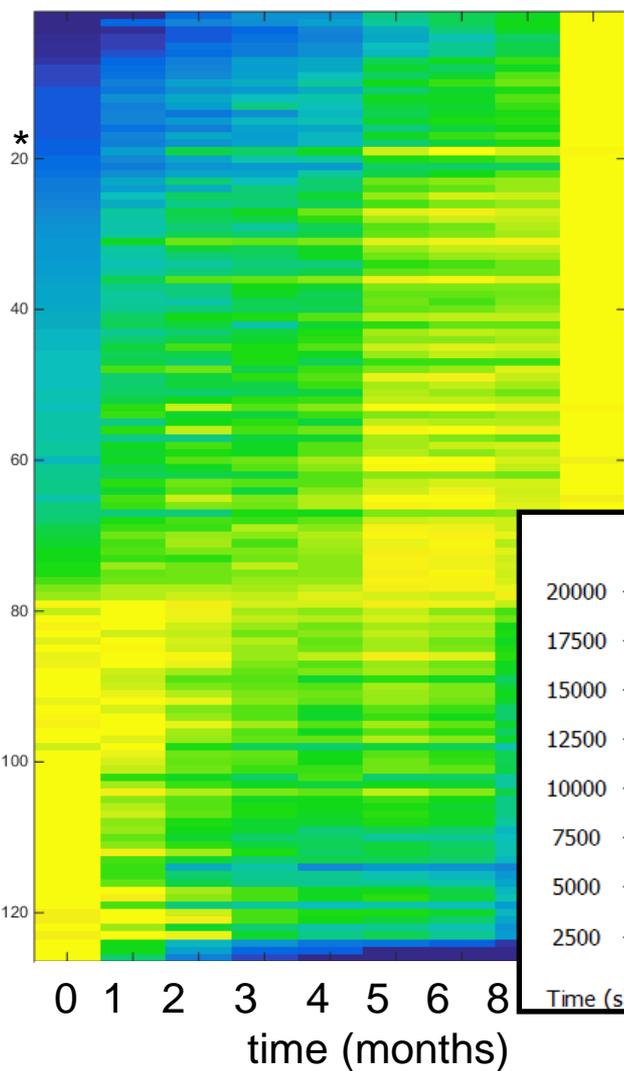
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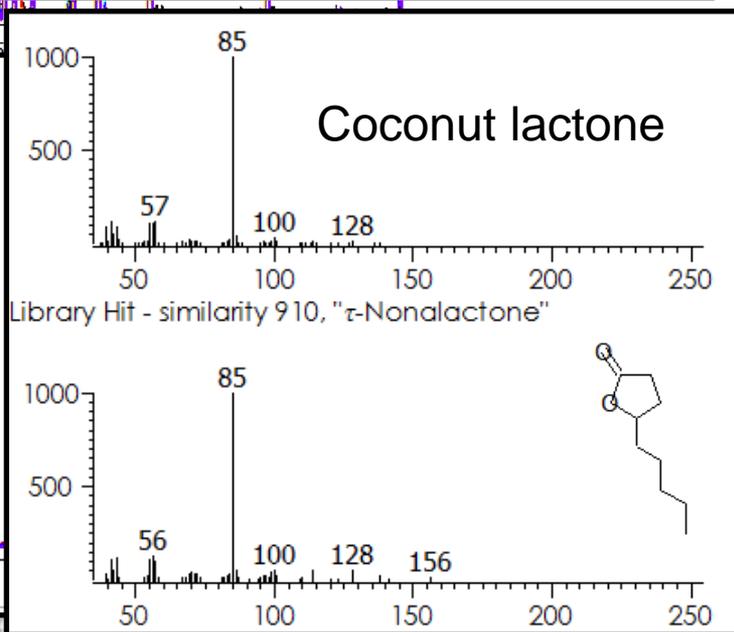
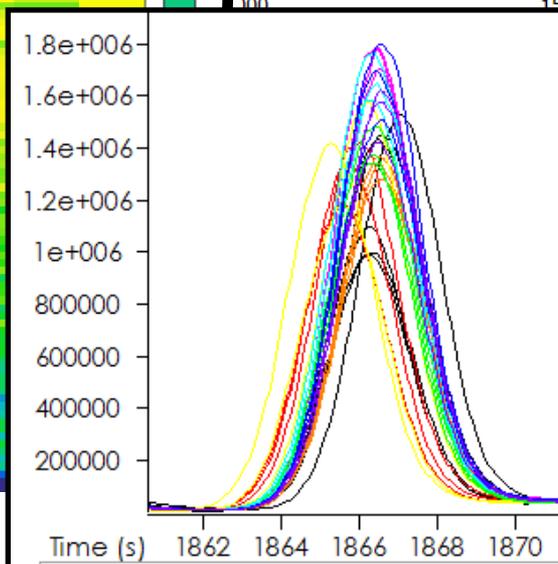
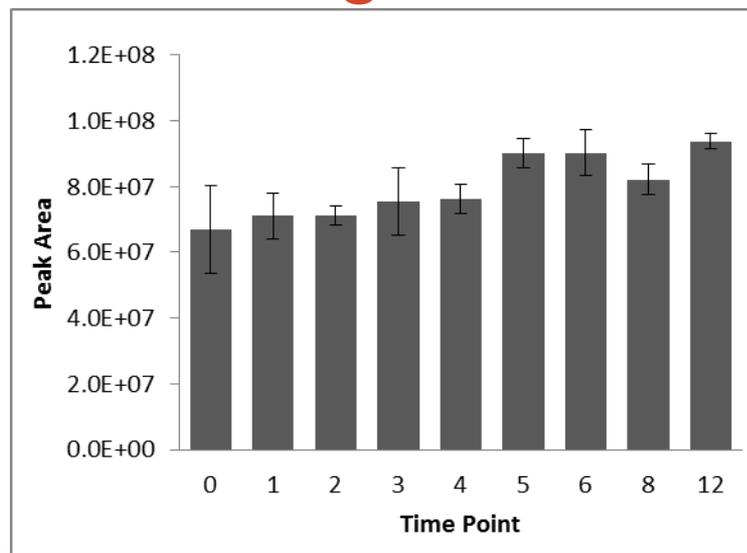
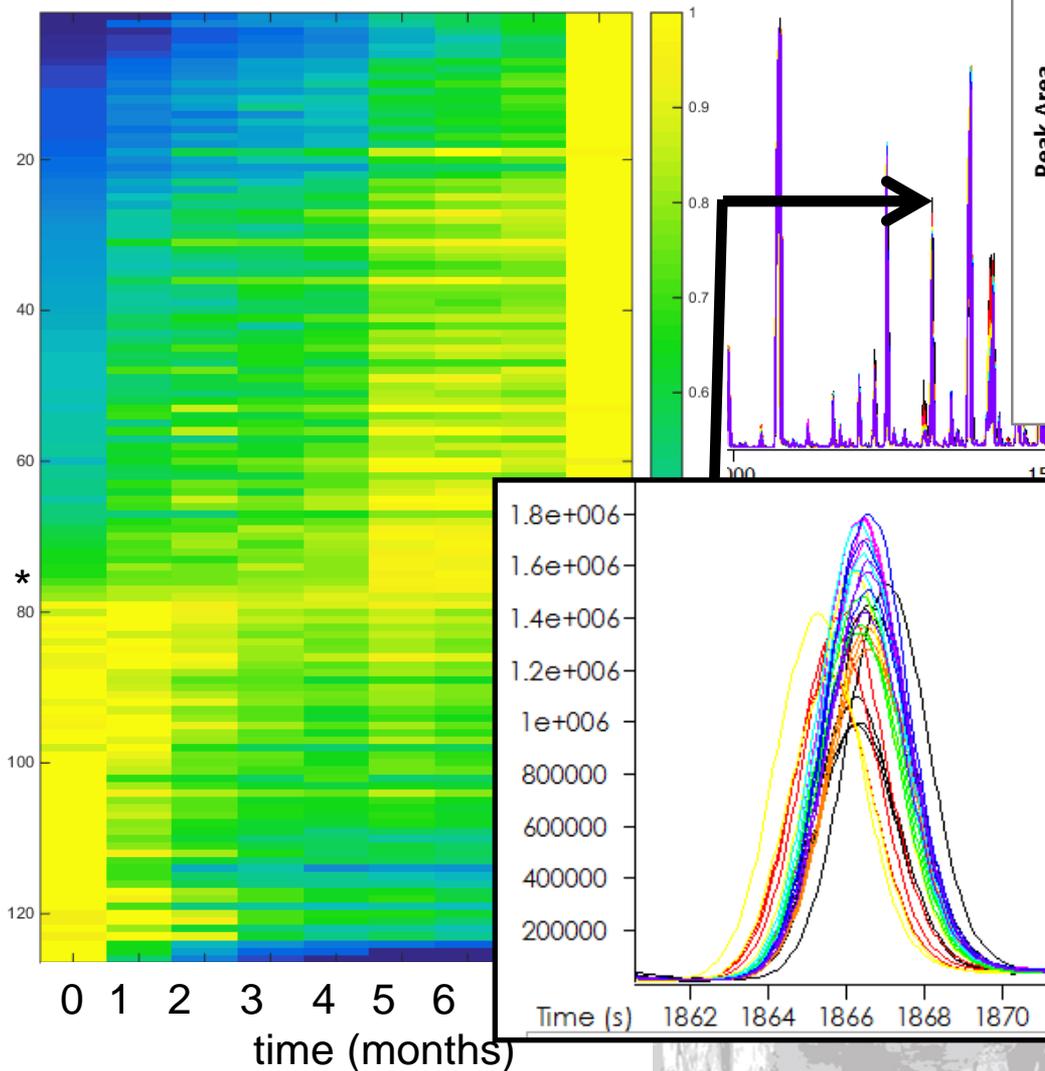
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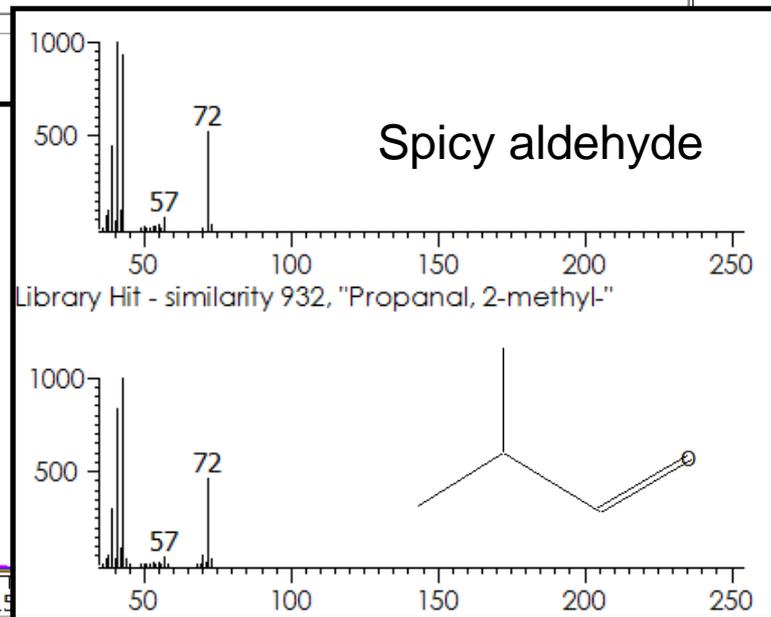
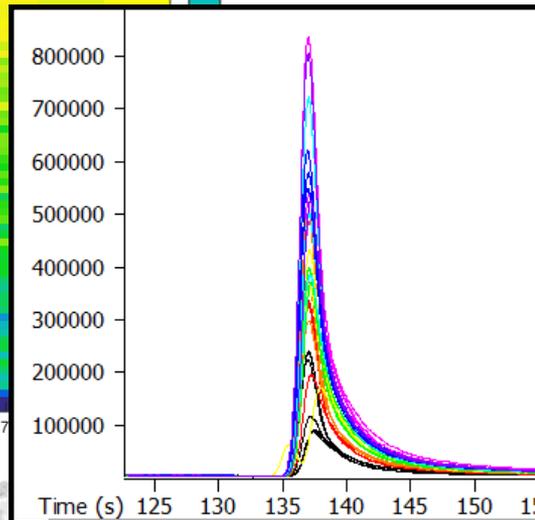
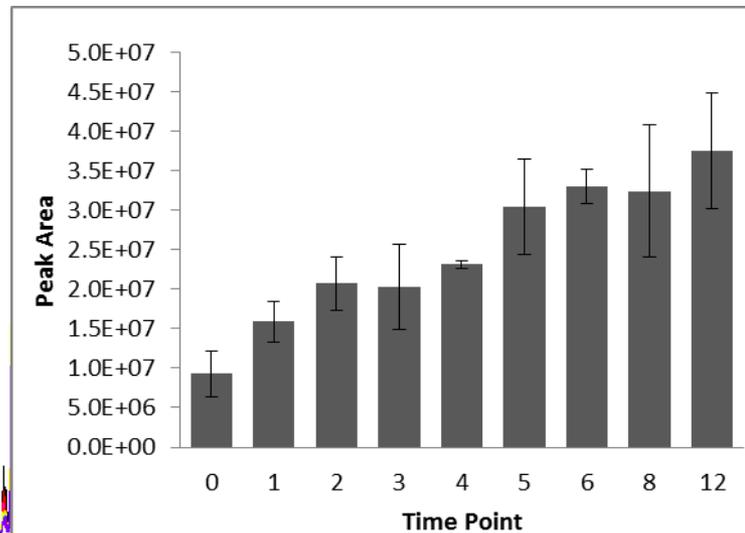
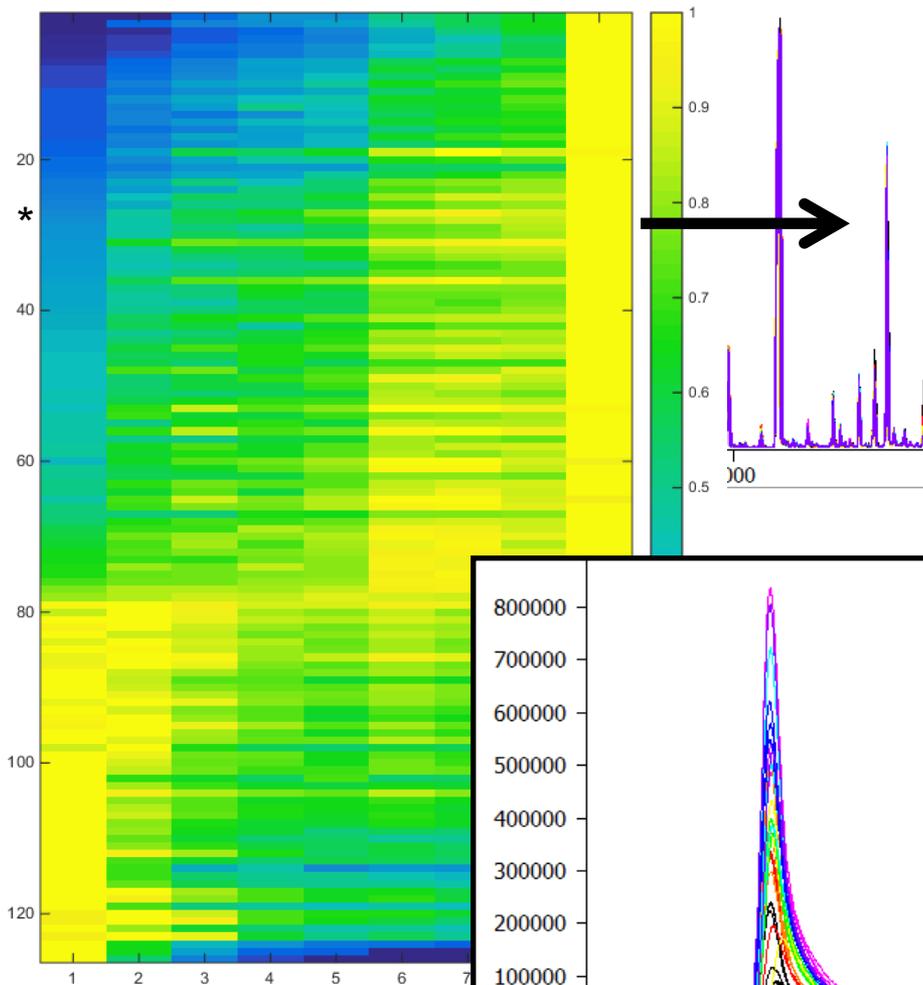
Analytes that Increase with Age



Analytes that Increase with Age

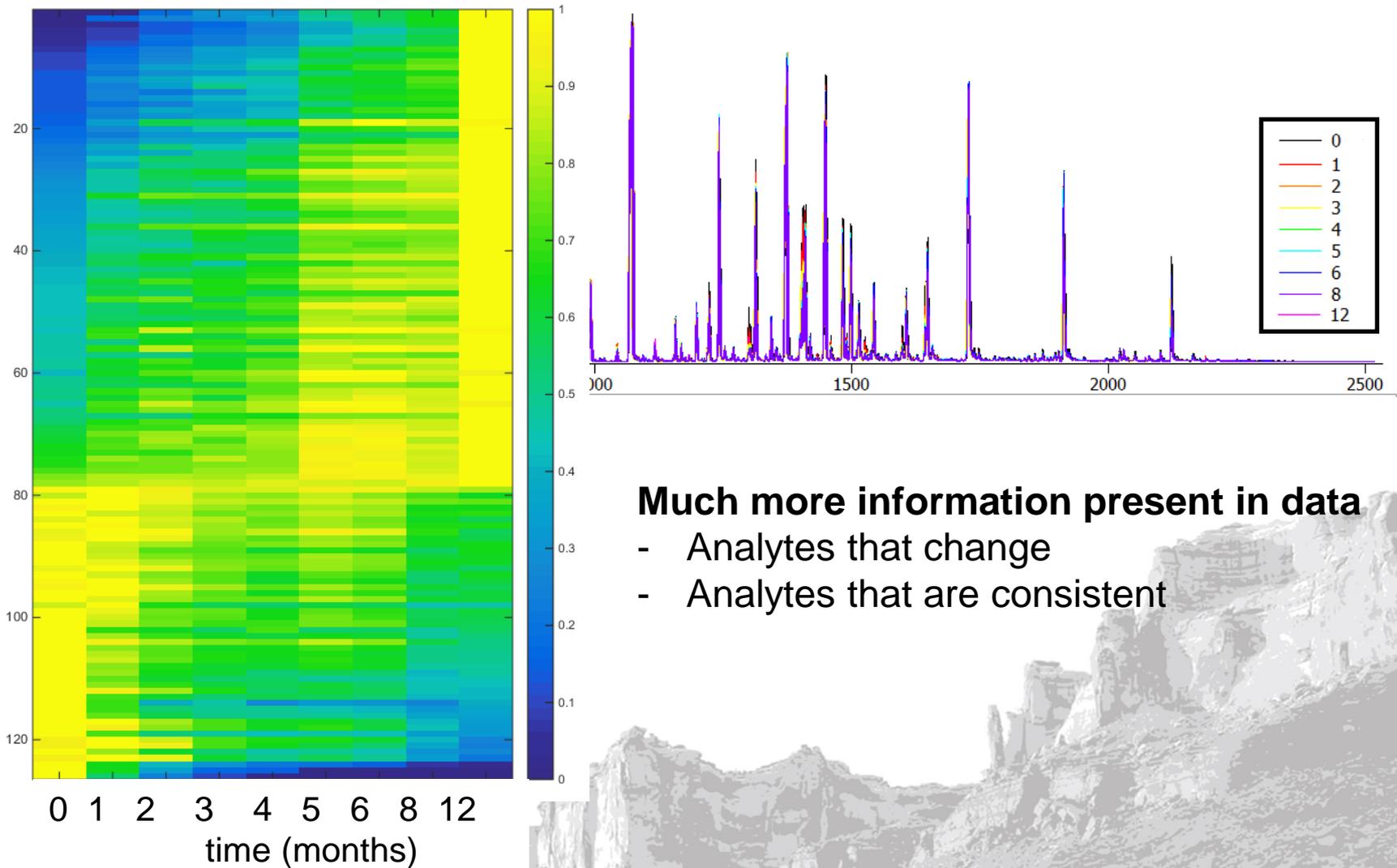


Analytes that Increase with Age





More Information



Much more information present in data

- Analytes that change
- Analytes that are consistent



Summary

- **GC-TOFMS is a non-targeted analytical technique that provides comprehensive data that can be mined for specific target analytes of interest and also reviewed for inherent trends and differences in the data without specifying target analytes in advance of acquisition for discovery analyses**

