The Good and the Bad Influence of Polyfunctional Thiols in Beer Hops; Is it Grapefruit or a Cat Box?

Application Scientist: Matthew Curtis

Quick History of Beer Hop Analysis:

Early life:

- In Europe there were about 20 varietals by 1900, which included Fruggle
    - 1904 was the start of cross breading (Many current hops have Fruggle in their family tree)
- 1819 was first description of volatile hop aroma
- 1895 the identification of myrcene, linalool, and geraniol was published
- 1956 first data collected on hop aroma with a GC
- 1964 GC/MS used for aroma identification

More current research:

- 2006 discovery and identification of 4-mercapto-4-methylpentan-2-one
    - Low concentration gives black current and box tree; high conc. cat urine
- 2009 Hanke, et. al. hop aroma interactions
    - Synergistic of aroma compounds

Willamette Valley Oregon; Coleman Agriculture

Half-Flower, Half-Amazing; Humulus Lupulus:

- There are both a male and female plant
  - The female flower is used for beer
  - Over 250 varietals today
- Lupulin glands provide acids and aroma
  - Over 500 aroma compounds
- Grown on trellises about 12 feet tall
  - Only about 1 foot is left after harvest
- After harvest the cones are separated from the vines and dried.
- Whole cones and pellets are used in beer
  - Whole cone is preferred but difficult to keep fresh
  - Some aroma compounds decrease by 50% in aluminum vacuum bags after 6 months at 20 °C
- Extraction of the essential oils and aroma compounds have created products for final beer additions

http://sicilianosmkt.blogspot.com/2016/08/homebrew-science-hops-part-i-alpha-and.html

Willamette Valley Oregon; Coleman Agriculture

Use of Hops in Brewing:

Bittering vs Aroma Hops

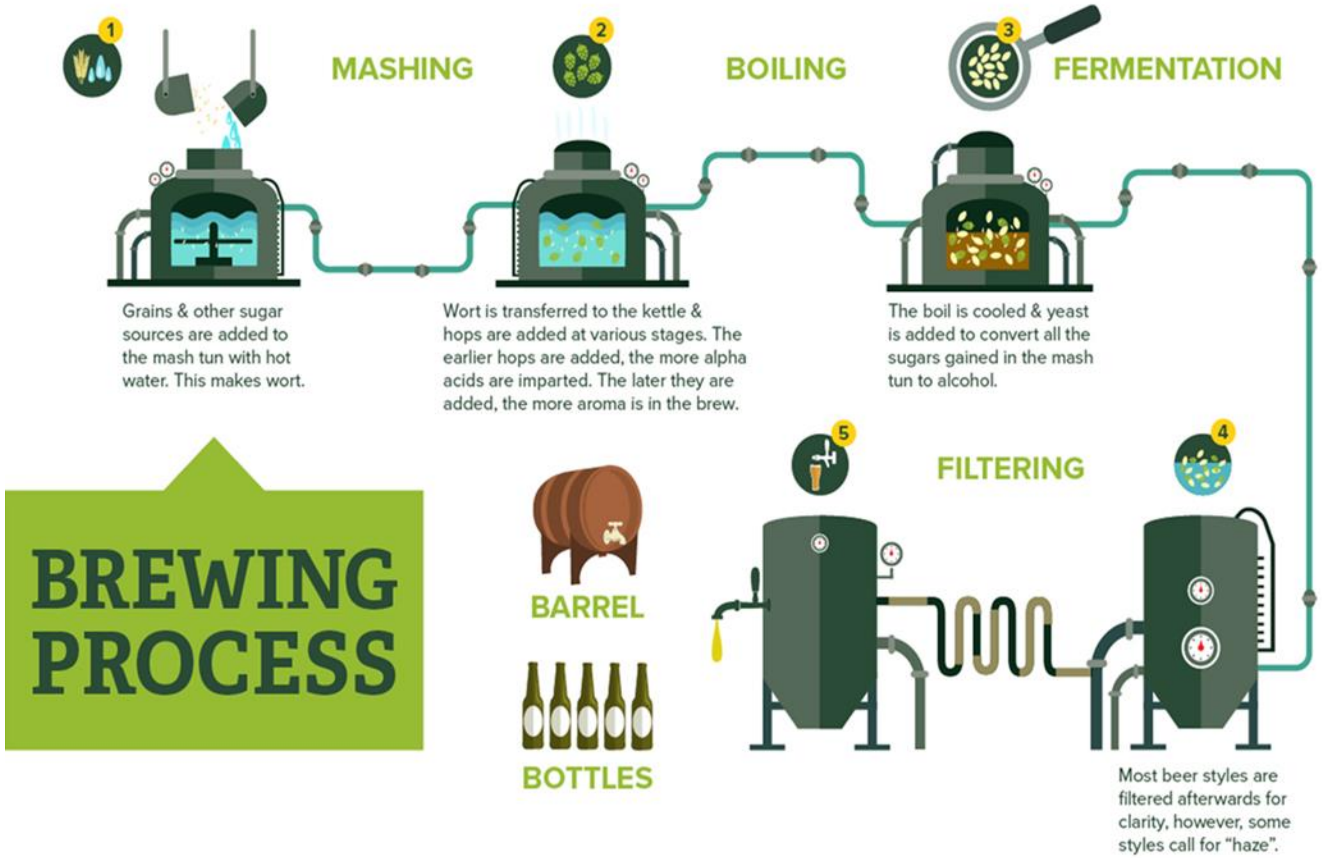
Bittering Hops:

- Added to wort during boil
- Heat extract alpha-acids
  - Tetrahydroiso (2x), Iso (1x), Dihydroiso(0.8)
- Counteracts sweetness from sugars
- Usually a mix of varietals
- Provide little to no aroma in the final beer

Aroma Hops/Dry-hopping:

- Added to fermenting or finished beer
  - Aroma without bitterness (might extract iso-acids)
- Usually a mix of varietals
- Extraction depends on hop form and hydrodynamics

- Total amount of hops varies with beer style
  - Lager 0.19 lbs/keg; Imperial IPA 3.8 lbs/keg



https://www.usahops.org/enthusiasts/brewing.html

If Only We All Had Room for Our Favorite Beer Kegs:

Beer Packaging:

- Best beer integrity is from a keg
- The classic glass bottle and a metal cap
- Brown provides best UV protection
  - Light reacts with α-acids and thiols
    - Skunky beer
  - UV film can be placed on green and clear
- Cap liners can extract aroma compounds
  - Always keep bottled beer upright
- Aluminum cans are the next best to kegs
  - Full UV protection
  - Lining can absorb aroma compounds
- Look for more craft beer in cans; it has already started

https://wettenimporters.com/news-events/beer-colored-bottles/

The Mixture Smells Better Than the Individual Compound:

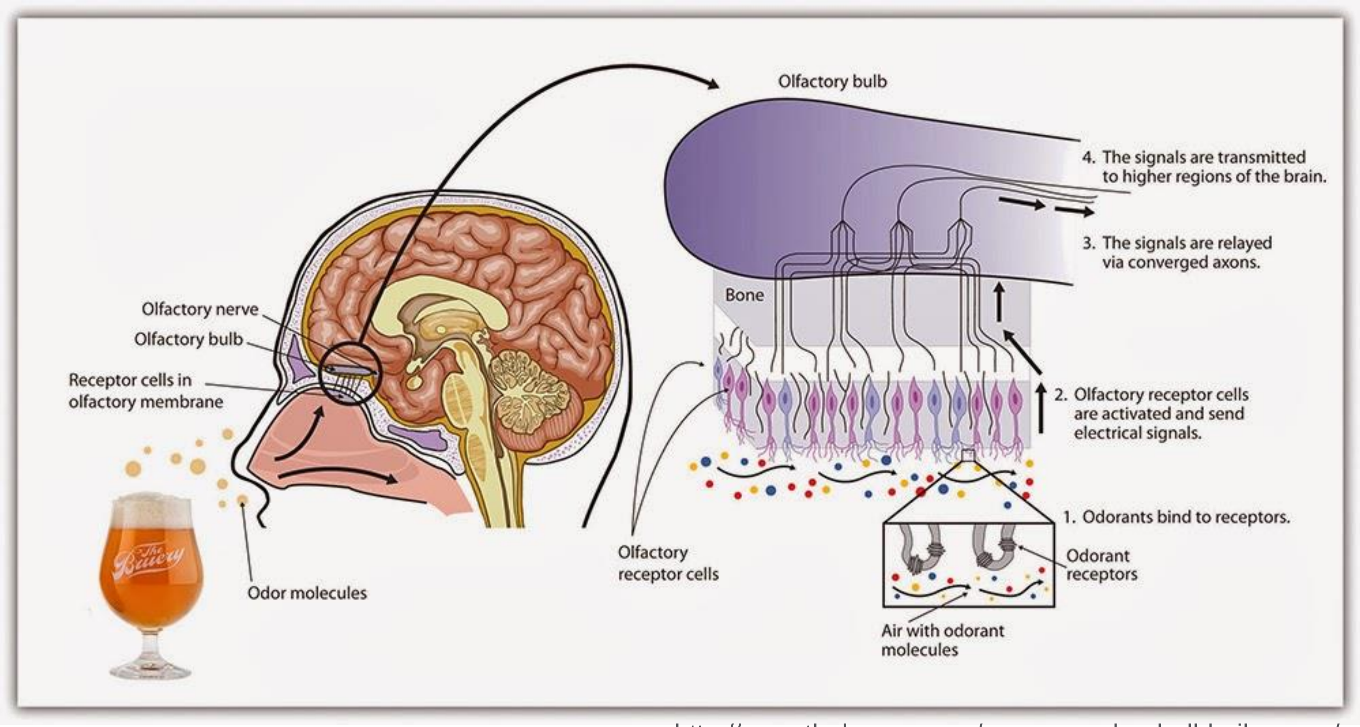
Aroma profiles are produced from a trained panel

Complexity of Smell:

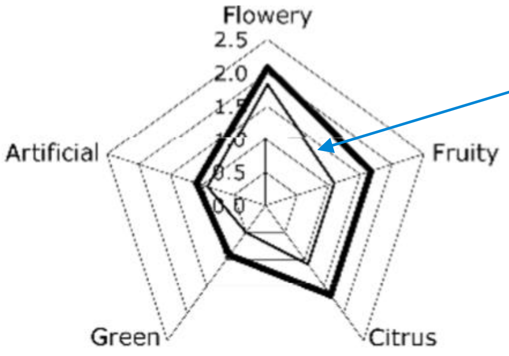
- Activated olfactory receptor cells sends signal to the brain
- 400 different odorant receptors
- Aroma is comprised of a pattern from the receptor signals
- High conc. of individual components can signal multiple receptors changing the pattern

Synergistic effects:

- Differences in aroma perception based on composition
- Citrus enhanced with the addition of β-citronellol and geraniol to linalool.



http://www.thebruery.com/sensory-school-all-hail-aroma/



Linalool (1000 μg/L) (CR-LF) + Geraniol (25 μg/L) (CR-LF) + β-Citronellol (20 μg/L)

(Takoi et. Al. 2010)

Polyfunctional Thiol Aroma Analysis:

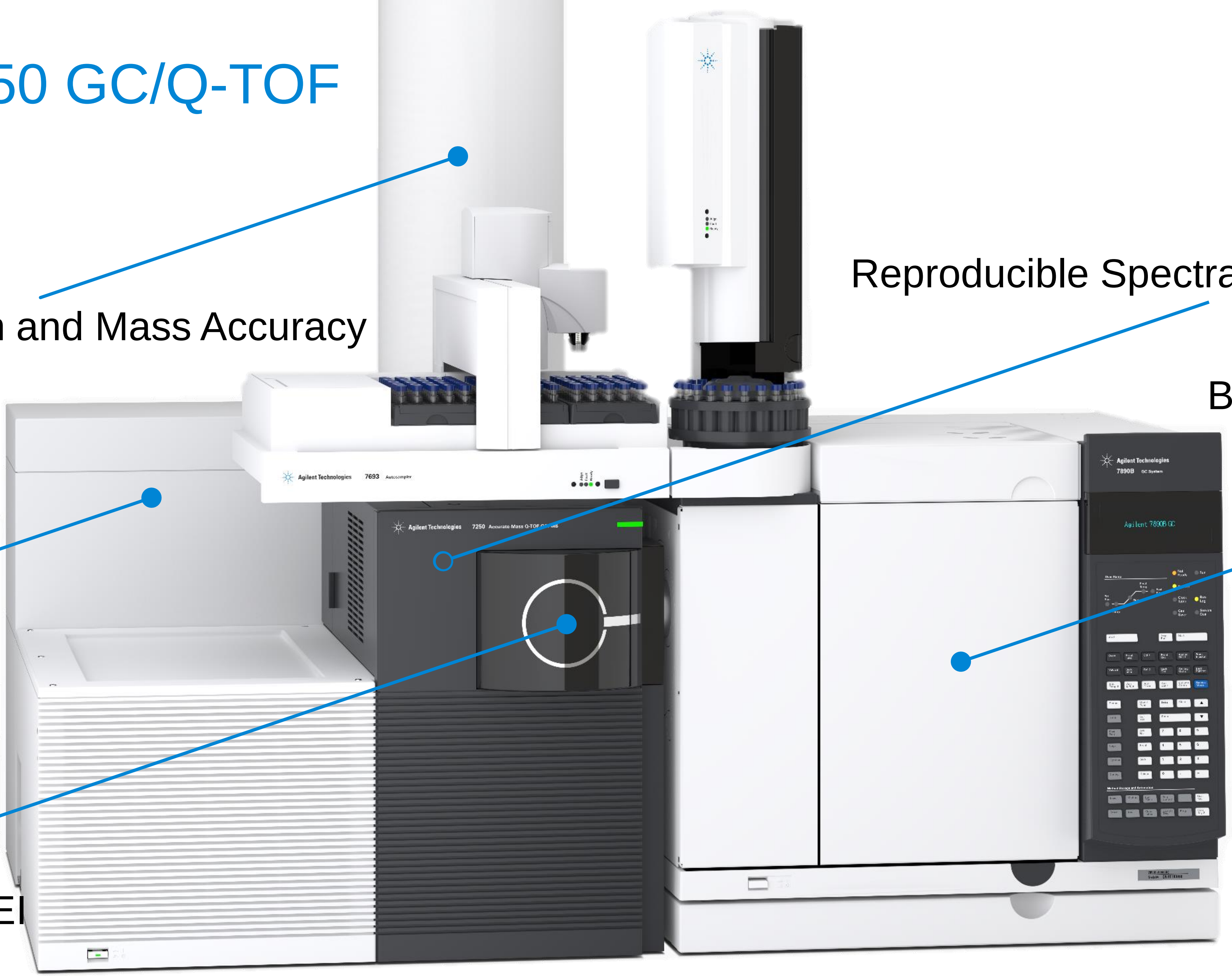
Difficulties in the analysis:

- About 3% of hops are aroma
  - Altered by genetics, cultivation, storage
- Hydrolysis, esterification, isomerization occurs during brewing
- Largest error is sample prep ~30%
- Thiols are normally 100 pg/kg threshold level
- Hg agarose gel has been used to isolate thiols for purification
- A QC level method needs to be created to reduce variation and increase accuracy
- Using GC-PFPD about 41 polyfunctional thiols have been identified.

Agilent QqQ GC/MS with Gerstel SPME


Agilent SCD on 7890B GC

The Agilent 7250 GC/Q-TOF



High Resolution and Mass Accuracy

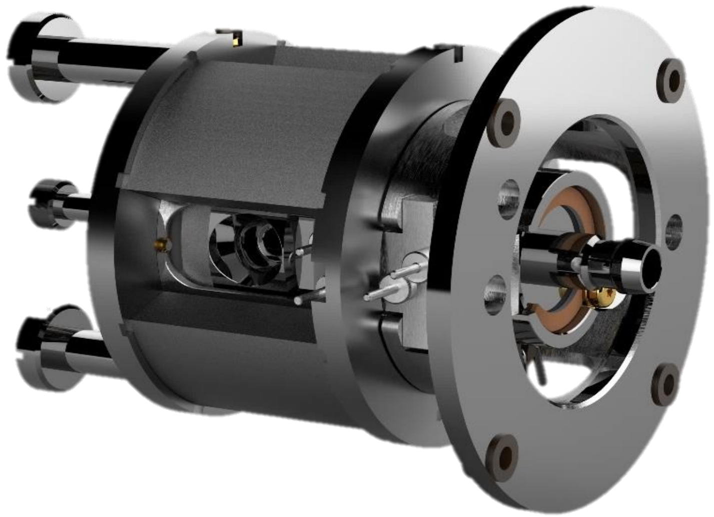
Reproducible Spectral Performance

Backflush-ready
Agilent
7890B GC

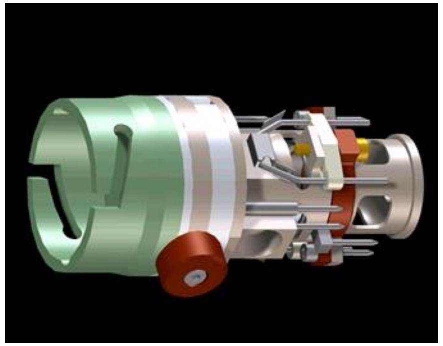
Simultaneous
High Resolution and
Wide Dynamic
Range

Sensitive Low Energy EI

The Agilent 7250 GC/Q-TOF



Proprietary
Low Energy EI Source



7200 EI Source

Based on the High Efficiency Source Optimized for Low Energy EI:

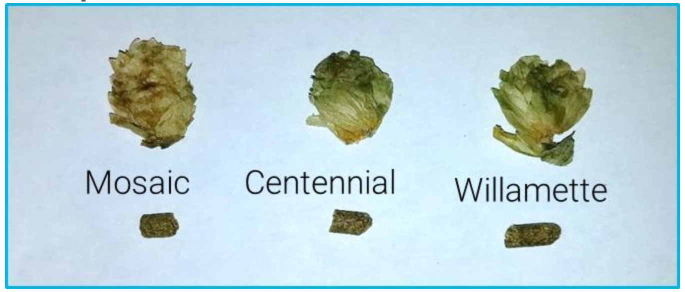
- Stronger axial magnet
- Modified lens geometry
- Centered filament

Instrument Specification:	
Resolving Power	>25,000 at *m/z* 271.9867
Mass Accuracy	< 2ppm RMS
EI IDL	<60 fg OFN (8 injections)
Electron Energy	5 – 200 eV
ToF Flight Path Length	3 m
Acquisition Rate	1 – 50 Hz

How Did I Collect This Data?

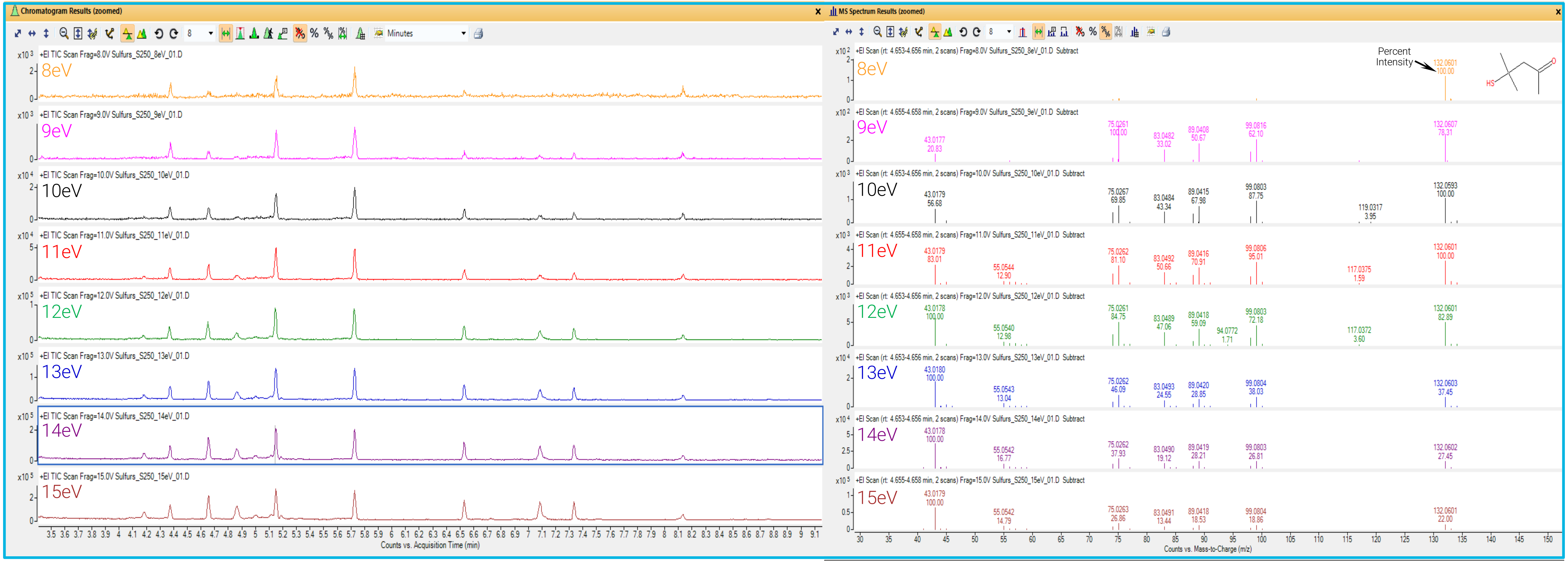
Sample Prep:

- Hop cone and pellets were purchase from More Beer (Los Altos, CA)
  - Centennial, Mosaic, Willamette, and Magnum
- 3g of hops were added to 300mL of 5 v/v% ethanol solution
  - The hop tea was left overnight
- The filtered tea was added to a headspace vial with 3g NaCl
- The Gerstel MPS Sampler was used for SPME automation
- Extraction was 50 min at 40 °C with a Supelco DVB/CAR/PDMS fiber
  - Desorption was 10 min



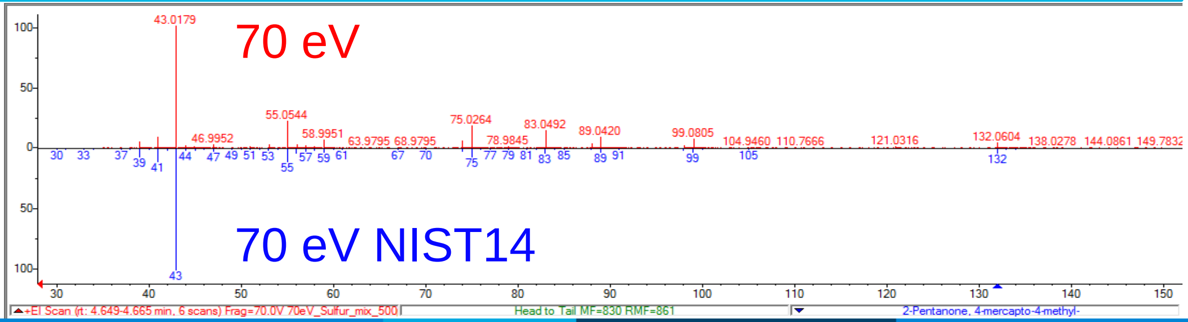
GC and MS Conditions:		
Column	DB-35ms UI, 30 m, 0.25 mm ID, 0.25 µm film	
Injection	Gerstel MPS SPME	0.75mm straight liner
Split	5:1 split	
Inlet temperature	270 °C	
Oven temperature program	40 °C for 1 min	
	20 °C/min to 50 °C	
	5 °C/min to 220 °C	
	220 °C hold for 5 min	
Carrier gas	Helium at 1.4 mL/min constant flow	
Transfer line temperature	250 °C	
Source temperature	250°C	
Quadrupole temperature	150°C	
Scan range	35 to 500 m/z	
Spectral acquisition rate	5 Hz, both centroid and profile	
Emission	0.8µA	
Ionization parameters used in the method		
12.5 eV low energy ionization		

Does Low eV Ion Source Work:



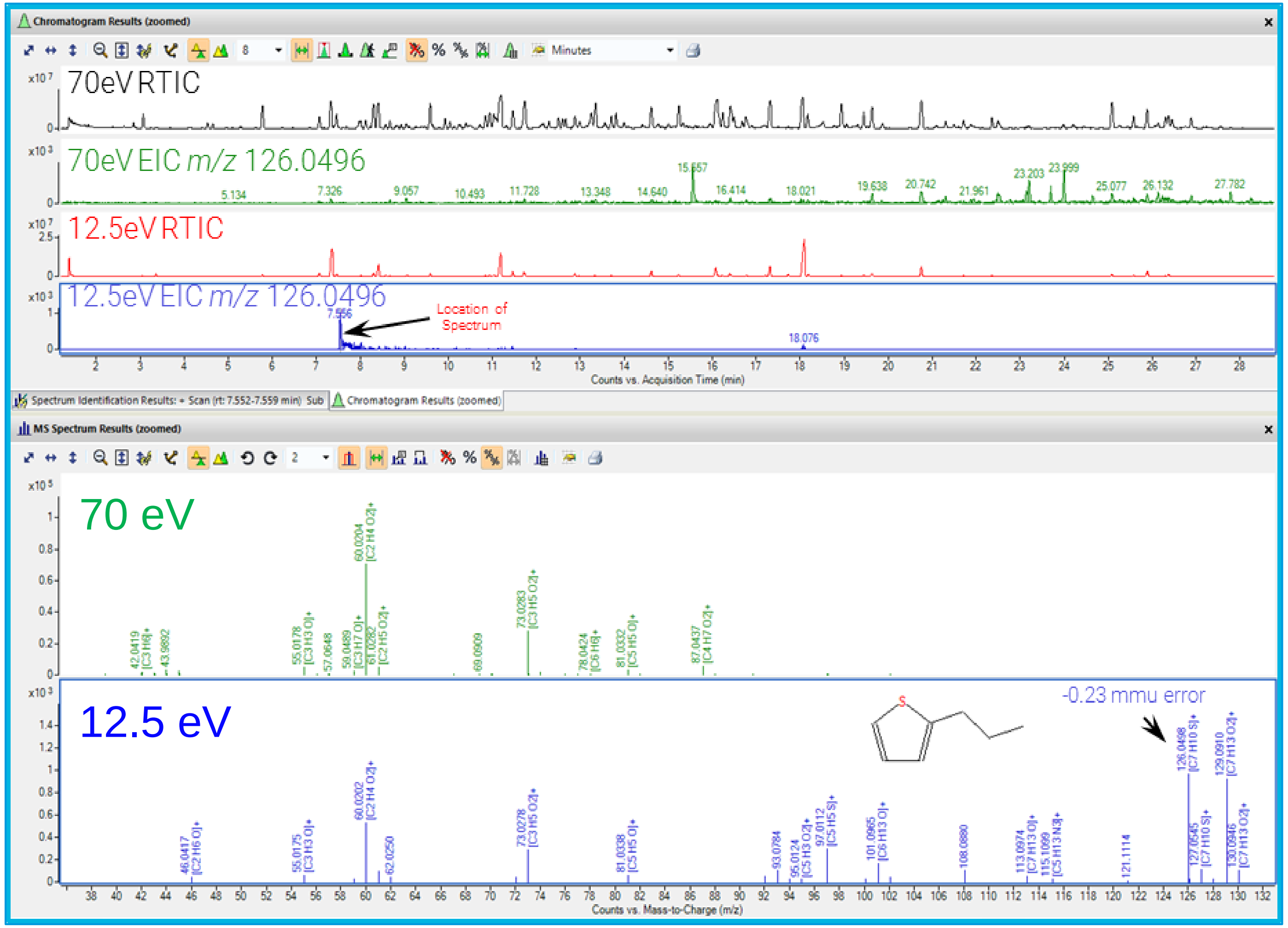
Polyfunctional thiol mix used for eV survey

4-mercapto-4-methyl-2-pentanone was selected for optimization



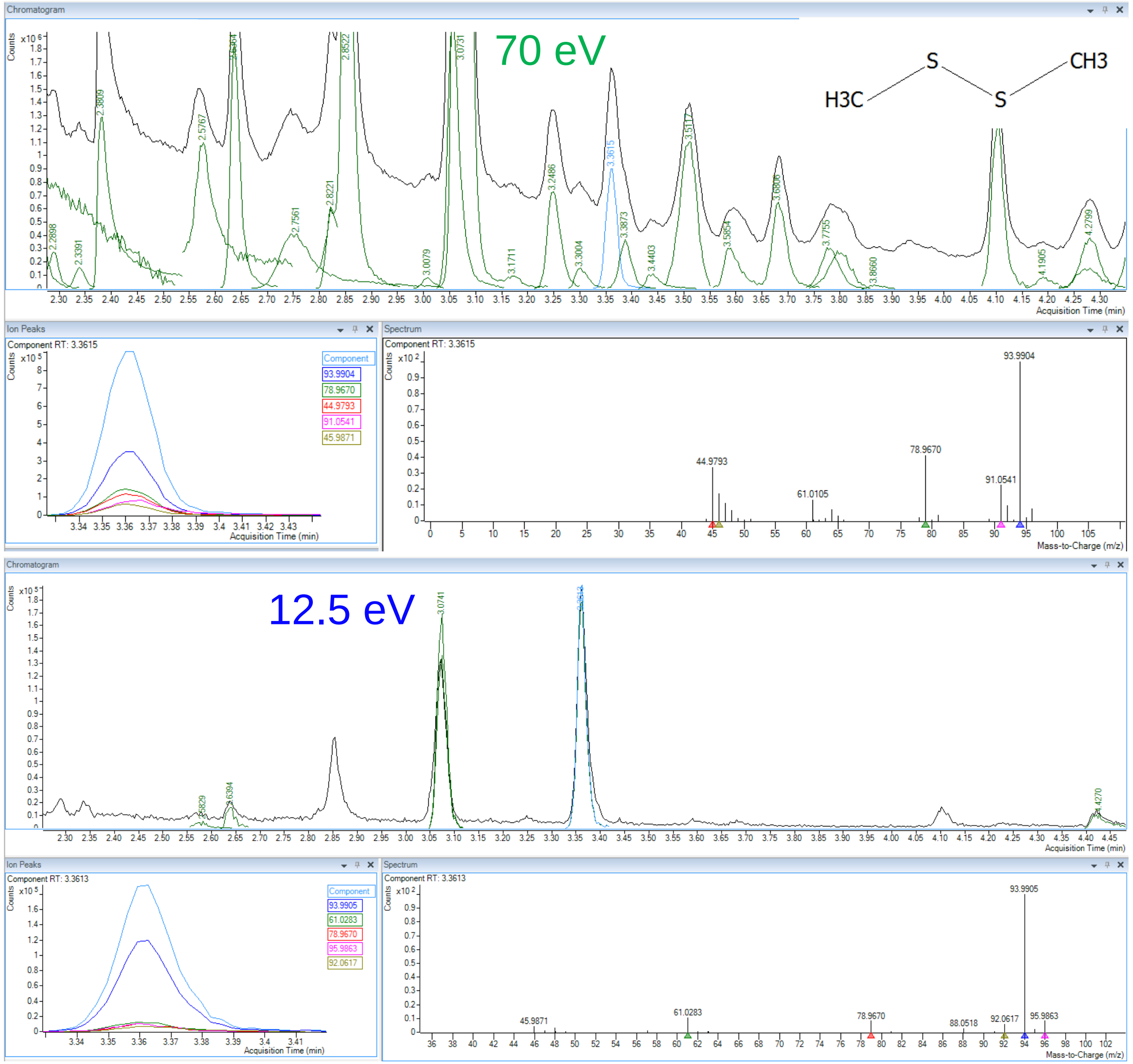
Does Low eV Ion Source Work:

- 70eV RTIC has a significant number of peaks, even with SPME sample prep
- Analysis of the 70eV data produced too much fragmentation to identify this thiol
- Even extraction of the exact mass did not easily locate the compound
- Low eV reduced the background and increased the molecular ion
  - Most of the 70eV spectrum was produced from the fragments of a terpene
  - At *m/z* 129.0019 in the low eV spectrum
- Isotope fidelity was preserved to aid in confirmation
- Aroma of roast beef and coffee



Does Low eV Ion Source Work:

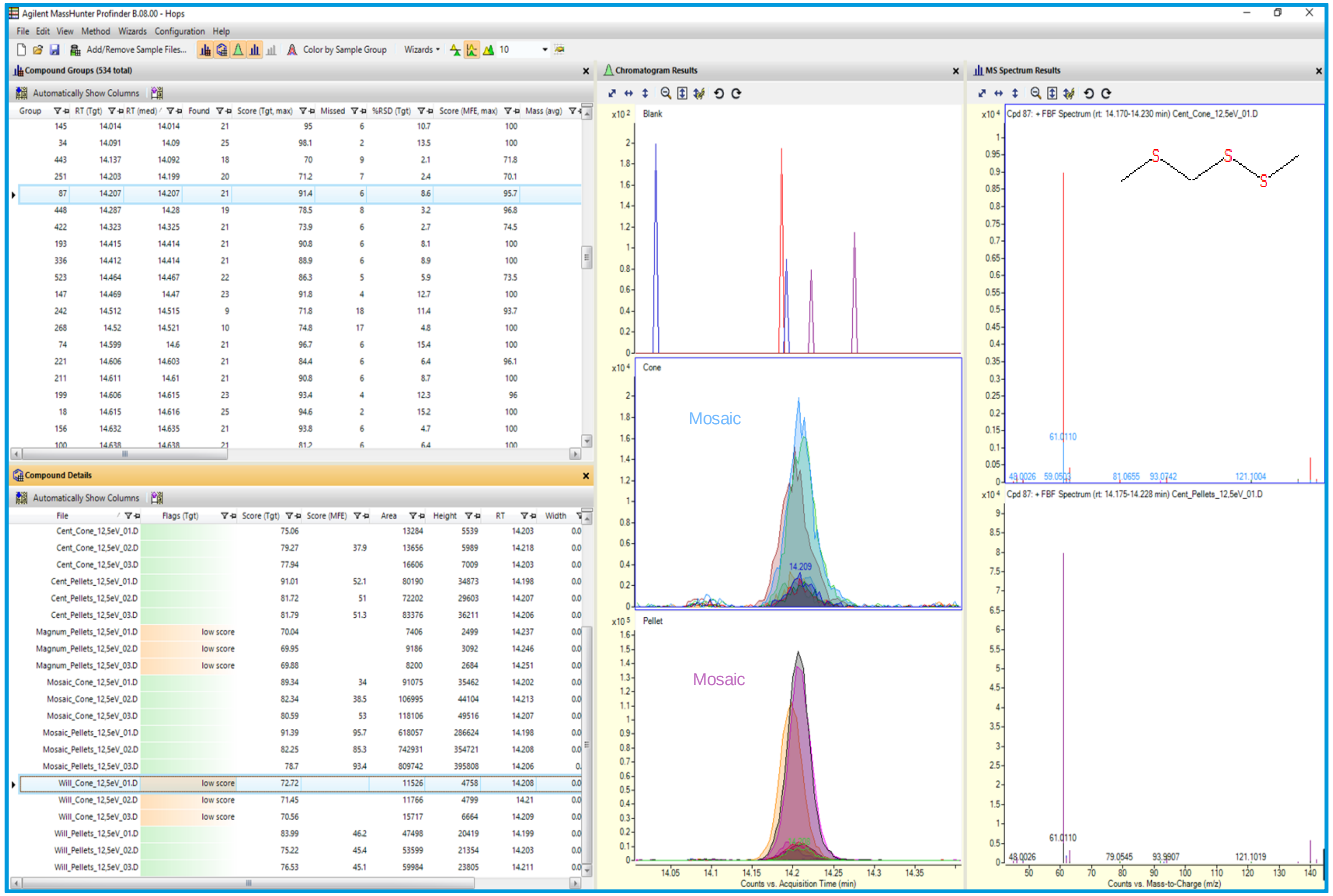
- 70eV RTIC has a significant number of peaks, even with SPME sample prep
- Top image is produced from SureMass peak detection
- This component co-eluted around a significant number of compounds
- Low eV reduced the background and increased the molecular ion
- Viewing the same RT window shows only a few compounds compared to the 70eV
- Almost all the ion current is produced by the molecular ion
- Dimethyl disulfide can enhance the fruity notes of beer, and additional truffle and black olive characteristics



Agilent

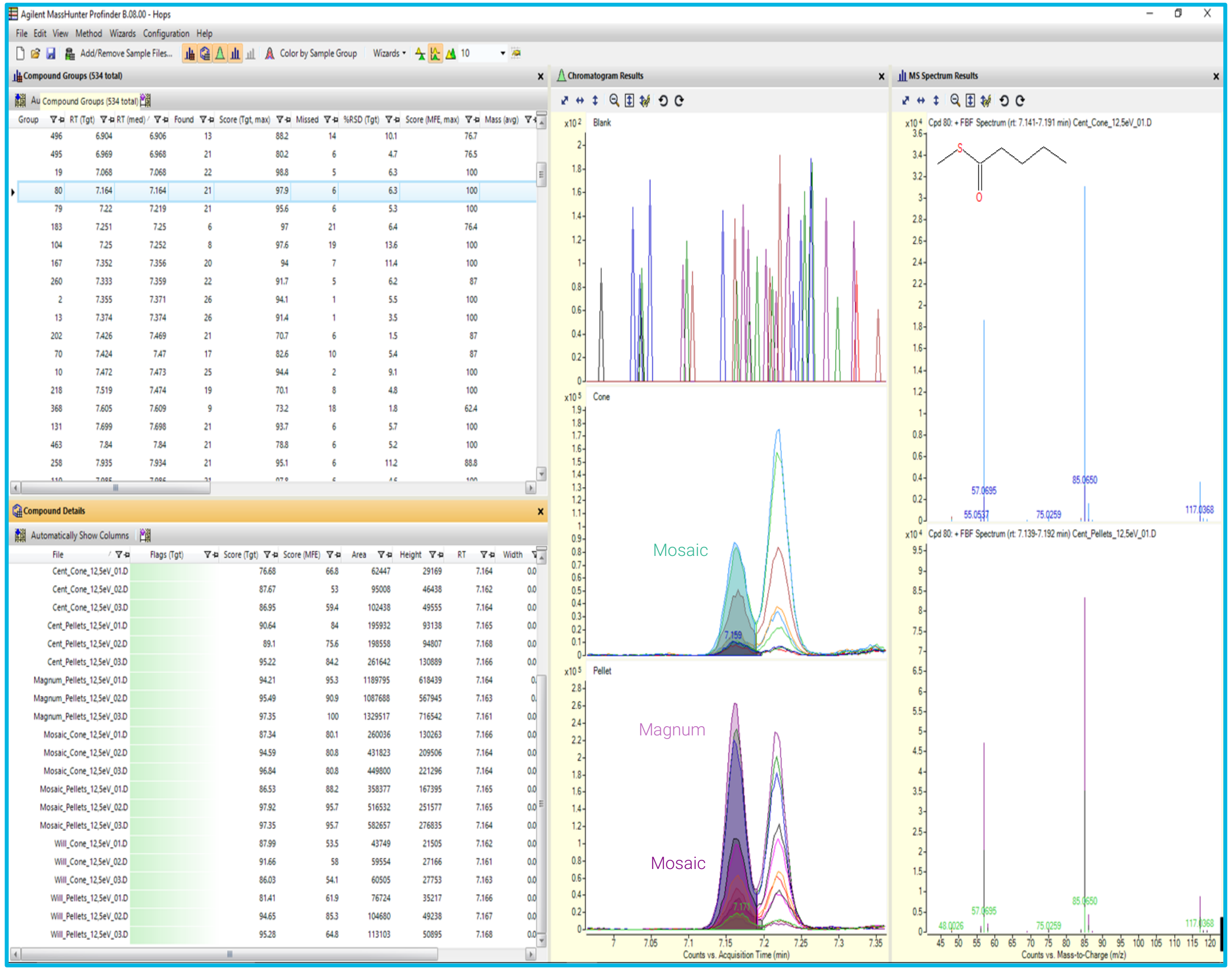
Does Low eV Ion Source Work:

- ProFinder Data Analysis for alignment and feature finding
- Molecular Feature Extraction followed by a recursive workflow reduced false negatives
- Samples were grouped by hop form for this image
- Trithiahexane was identified in larger concentrations for mosaic hops independent of hop form
    - The pellets had a higher concentration than the whole cones
    - This compound produces a onion aroma



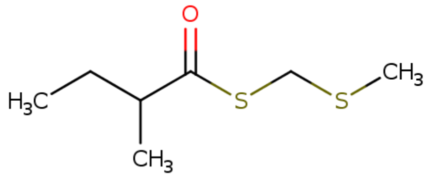
Does Low eV Ion Source Work:

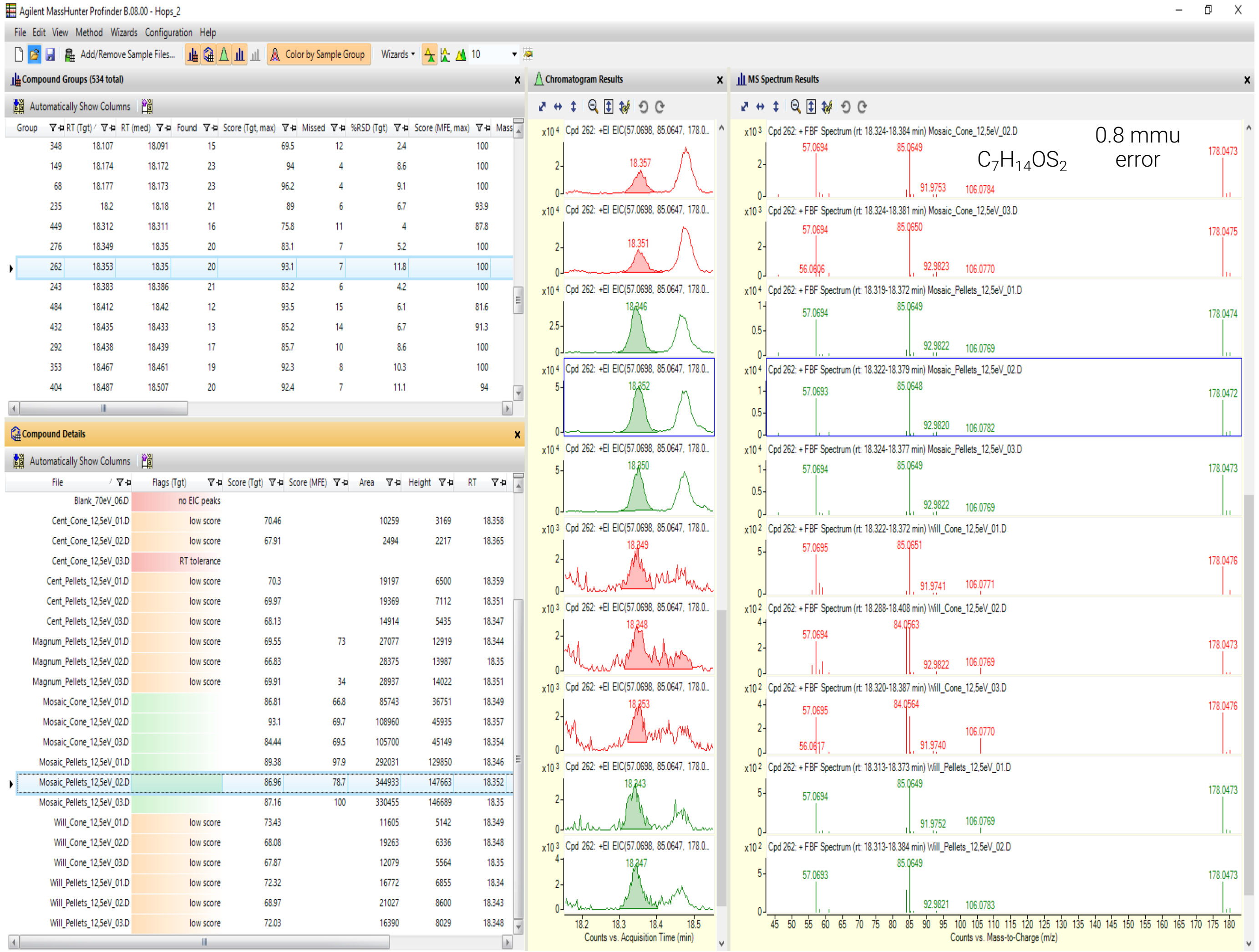
- ProFinder Data Analysis for alignment and feature finding
- Quickly scan through data to find components of interest
- Samples were grouped by hop form for this image
- S-methyl pentane thioate was identified in larger concentrations for mosaic cone and magnum pellet
    - The extracted peak to the right is a structural isomer
    - This compound produces a cheesy, mushroom, dairy aroma



Does Low eV Ion Source Work:

- ProFinder Data Analysis for alignment and feature finding
- Two di-sulfur containing compounds were observed in low eV ionization
- Mass accuracy and accurate isotopes were used for the confirmation of the elemental composition
- The highest concentrations were found in the mosaic cone and pellet
- Methylthiomethyl 2-methylbutanethiolate has been isolated from hop oil
    - Cabbage aroma

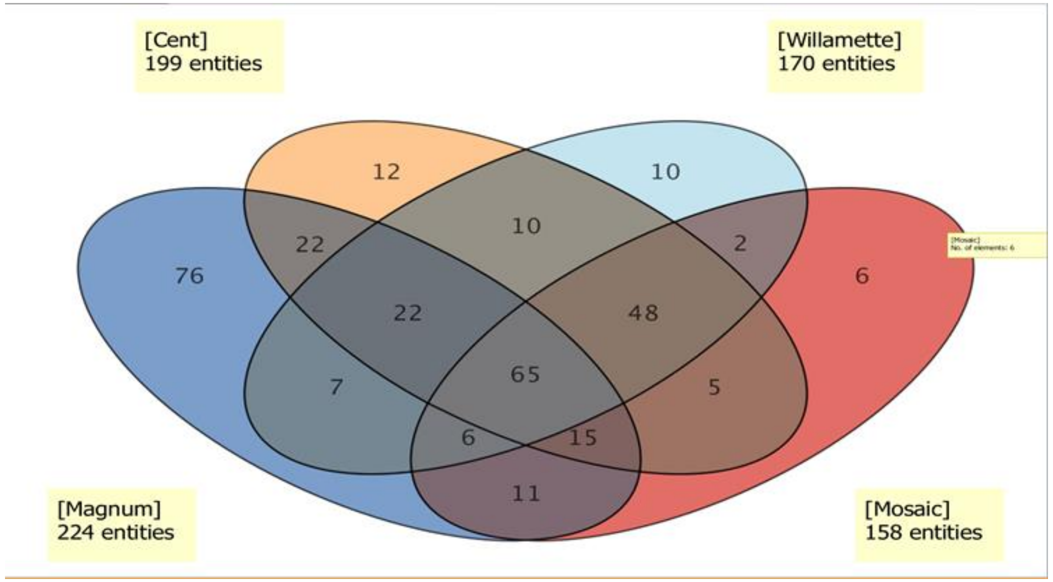




What to do With All of the Data:

Mass Profiler Professional:

- Too many components to spend time on the non-significant
- Send all of the Profinder found components to MPP for statistical analysis
- The data can be grouped and visualized to identify the components of interest
- All samples were normalized with an ISTD (2-bromo-3-methylthiophene)
- The Venn diagram was produced using all components with a >2 fold change between the samples
  - This included more than just sulfur compounds
  - Cone and pellet data were combined so only the hop varietal differences were observed
- Additional sensory data could be added to help identify the aroma changes with the instrumental data

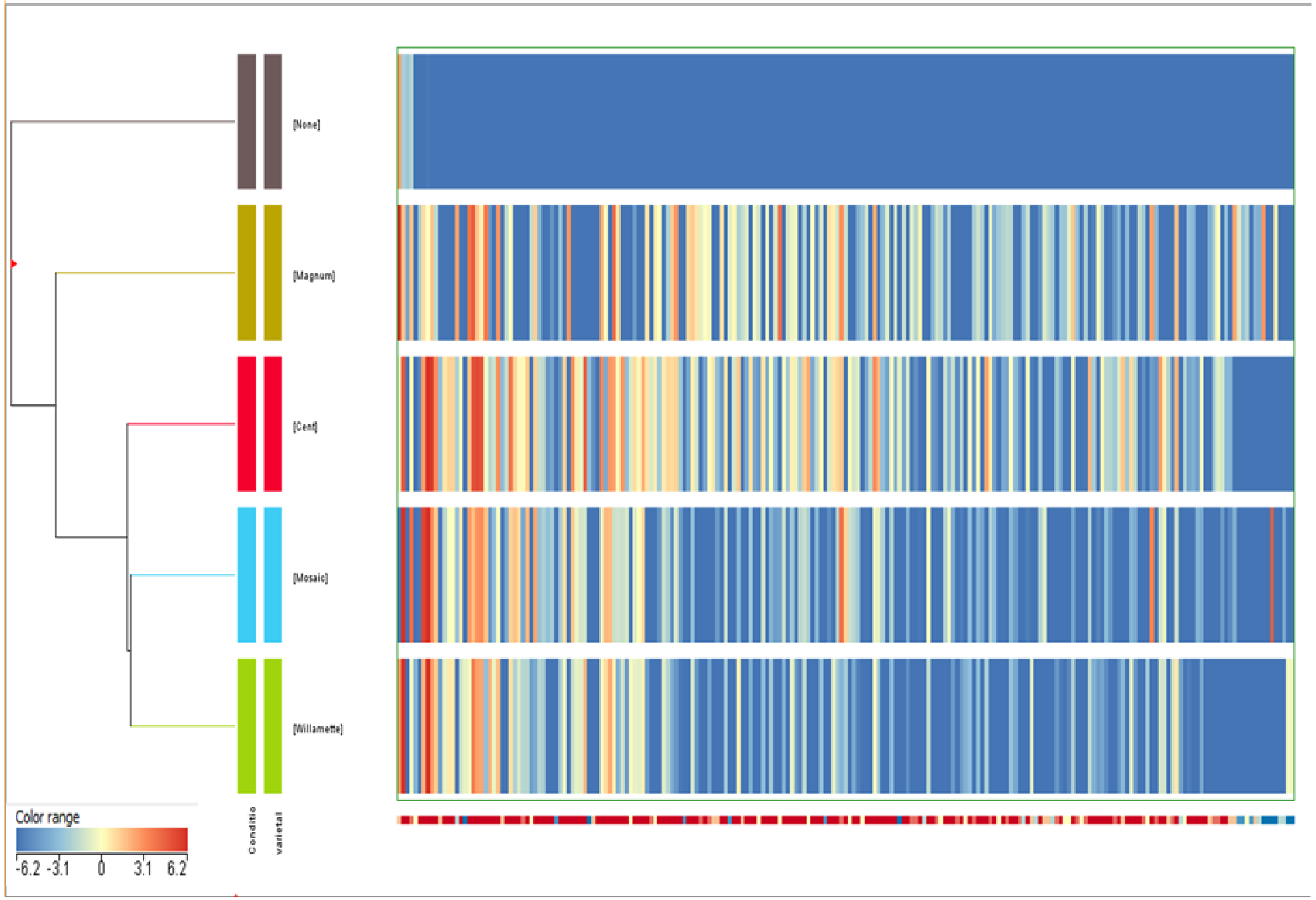


- About 65 components were shared by all four hops
- Mosaic, Willamette and Centennial share 48 components the most of any three

What to do With All of the Data:

Mass Profiler Professional:

- Clustering heat maps provide a quick visual of intensity differences
- The three technical replicates were averaged
- The comparison of components was set to observe the differences between the whole cone and pellets
- The bars with more “red” color show a higher response in the whole cone compared to the pellet
- The whole cones show more intensity for the early eluting, high volatility components
  - This makes sense since there was minimal processing of the hop

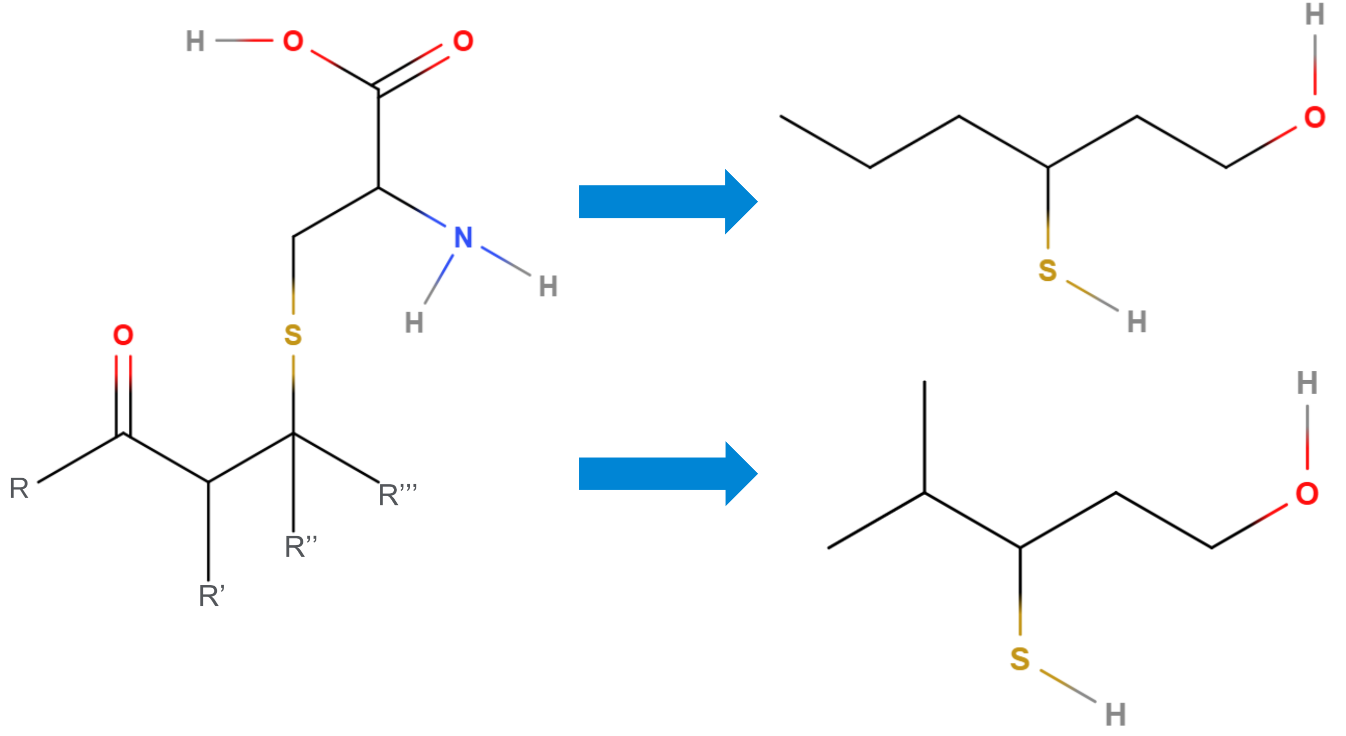


Future "Work" (Analyze Beer to Compare with Hops):

2 for Me; 1 for SPME

Analysis of beer with low eV:

- During the brewing process additional aroma compounds are released that are not observed in hops
- Some aroma compounds are produced from enzymatic reactions when hops and yeast are together
- S-cysteinylated and S-glutathionylated thiol precursors can enhance the polyfunctional hop aroma
- This process also produces new polyfunctional thiols not observe in just hops
- Additional sample prep optimization and GC configuration will also be explored
    - Possible use the dynamic headspace from Gerstel to perform FRET



(Takoi et. al. 2009)

Conclusion:

One Step Closer to Tasting

- The work is not done, with new varietals and new brewing techniques
- Low eV ionization is promising in the selective ionization of polyfunctional thiols
- Reduction of the background matrix provided enhanced signal for some of the polyfunctional thiols
- High mass accuracy and accurate isotope ration provided confidence in identifications
- Mono-, di-, and tri- thiol compounds were identified in the hop cones and pellets
- Statistical significance software allowed for focused data analysis of important components
- For the tasting, try to identify any fruity, earthy, citrus notes before and after you take your sip. An empty glass can provide better aroma profile