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Analysis of Sulfur Containing Compounds in Heavy Matrices using Low Energy Electron Ionization

Matthew Curtis; Jennifer Sanderson
Agilent Technologies, Santa Clara, CA

Introduction

Sales of craft beer has been on a steady increase, with a 6.2% increase last year. The number of breweries in the United States grew 16.2% from 2015 to 2016 and is expected to grow even more this year. The popularity of more flavorful and unique beer has created the need for the analysis of beer hops for optimal flavoring. There are about 80 commercial varieties of hops available to brewers, but similar to wine, the terroir can affect the chemical composition of the hop cone. Polyfunctional thiols are significant in defining the hop's character, but difficult to identify in hops due to their low concentration compared to terpenes and other aroma components. Two forms of hops (whole cone and pellets) of several varietals used by brewers were analyzed to identify possible differences between forms. This work discusses the utility of low energy ionization on the novel Agilent 7250 GC/Q-TOF as a possible solution to the fragmentation of the polyfunctional thiols as well as suppressing the hydrocarbon rich background.

Figure 1: Whole cone and pellet samples

Figure 2: Agilent 7250 GC/Q-TOF

Experimental

Sample Preparation:

Neat standards for several different polyfunctional thiols, headspace grade water and absolute ethanol were purchased from Sigma Aldrich (St. Louis, MO). The hop whole cone and hop pellet samples (figure 1) were purchased from More Beer (Los Altos, CA). These included Centennial, Mosaic, Willamette, and Magnum hop varietals. For sampling, 3g of hops were placed in 300mL of a 5% ethanol solution (by volume) to create a "hop tea." The suspension was set in the refrigerator overnight, filtered, and 10mL was added to a 20mL amber headspace vial. 3g of NaCl was added along with 2-bromo-3-methylthiophene (ISTD).

SPME Sampling:

The samples were prepared for injection using the Gerstel MPS Autosampler, utilizing Maestro.

The vials were incubated for 2 mins at 40°C prior to a 50 mins extraction using the Supelco DVB/CAR/PDMS 23 ga fiber. The fiber was injected into the Agilent MMI with the Merlin MicroSeal, for 10 mins. The GC parameters can be found in table 1.

Each sample was prepped in triplicate and the sample injection sequence was randomized to minimize replicate sample variances.

Table 1: Agilent 7250 GC/Q-TOF; 7890B GC Parameters

GC and MS Conditions:		
Column	DB-35ms UI, 30 m, 0.25 mm ID, 0.25 µm film	
Injection	Gerstel MPS SPME (pink)	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	
Spectral 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 (after optimization)		

Results and Discussion

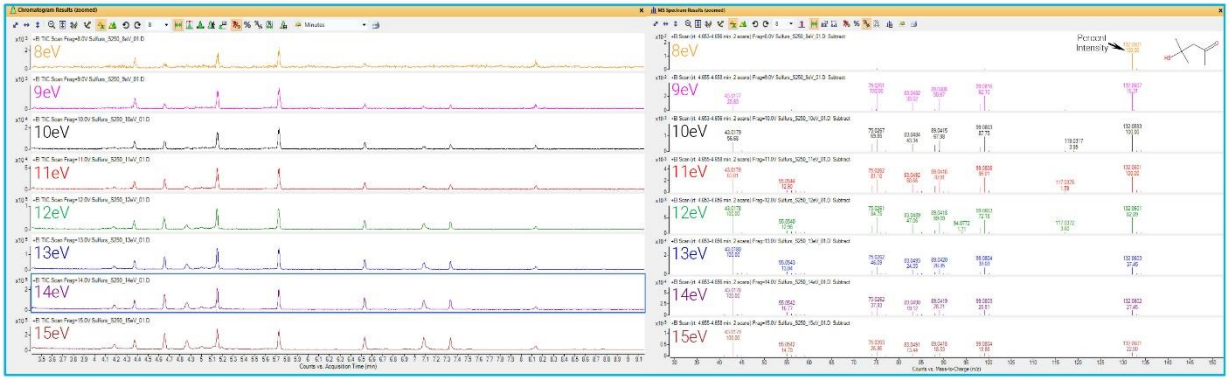

Figure 3: Sulfur standard eV survey to identify the optimal ionization energy.

Figure 4: Comparison of 70eV and 12.5eV for a co-eluting component.

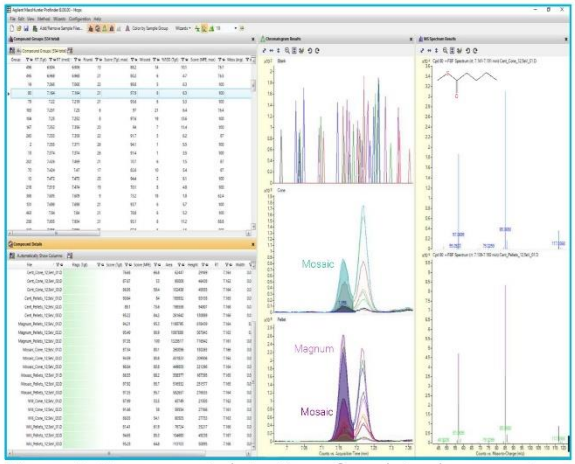

Figure 5: Component detection of analytes by grouping samples.

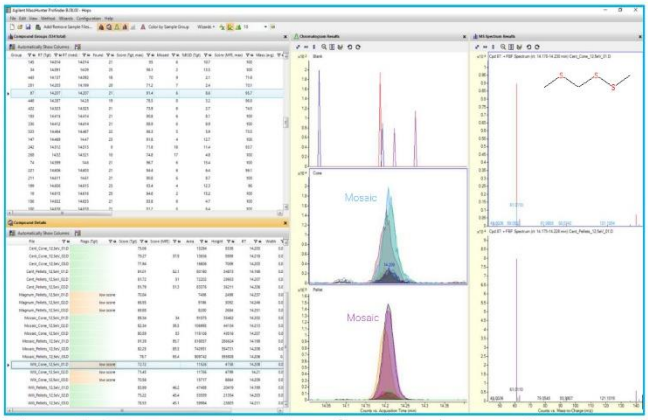

Figure 6: Component detection of analytes by grouping samples illustrating differences in varietals.

Discussion of figures.

Multiple injections were performed to vary the ionization energy and select an energy where the spectrum was tilted towards the high *m/z* range without a significant loss in sensitivity (Figure 3). After optimization, it was observed that one of the analytes was not detected due to the high degree of fragmentation and low concentration. The low energy eV analysis provided a molecular ion and isotope for confirmation (Figure 4). Figure 5 and 6 were produced from Agilent's Profinder B.08 using Molecular Feature Extraction to detect components within a chromatogram. The samples were grouped based on the type of hop form (cone vs pellet). Each varietal is overlaid with the group to illustrate the difference in intensity and a quick identification of significant components.

Results and Discussion

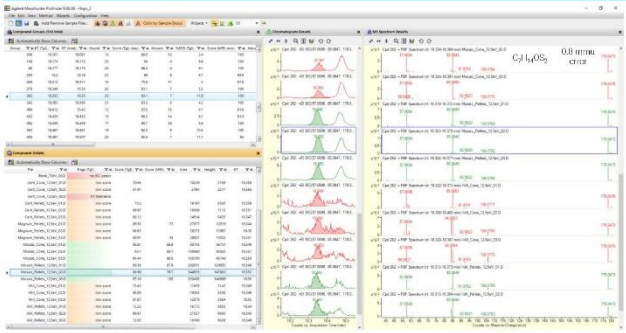

Figure 7: Molecular ion information for two isomers of $C_7H_{14}OS_2$ only observed in low energy ionization.

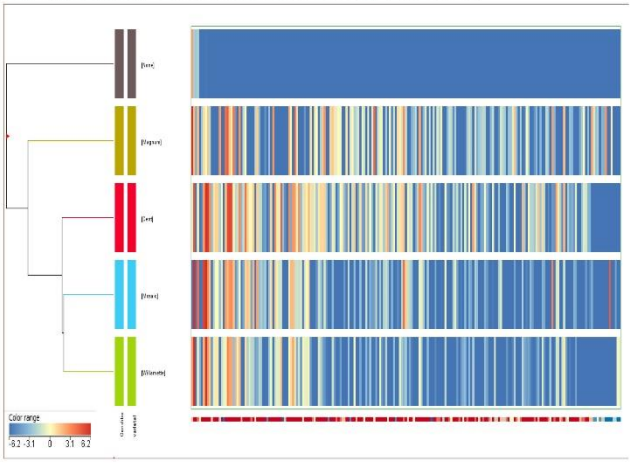

Figure 9: Clustering heat map illustrating the entities with an increased intensity when compared to the pellet.

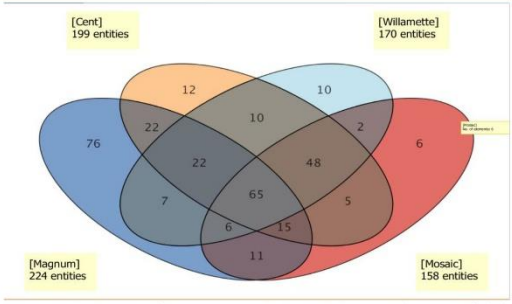

Figure 8: Venn diagram using entities with a >2 fold change.

Discussion of figures.

Figure 7 demonstrates the identification of two isomers of a di-sulfur component with molecular ion information retained. The following images were created in Agilent's Mass Profiler Professional after the analysis of all samples with Profinder. All of the samples were normalized to the ISTD (Bromo-thiophene) prior to statistical significance analysis. The Venn diagram (Figure 8) was produced using a >2 fold change requirement to show the diversity of the different varietals, 65 entities were found in all four samples. The last image was produced by averaging the three replicates for each sample and comparing the differences in intensity of entities with respect to the whole cone and the pellet forms (Figure 9). The more "red" a bar in the heat map a higher intensity was observed in the whole cone for each varietal.

Future Work.

Continue to optimize the extraction with SPME fiber selection and extraction parameters. Purchase additional standards to create a low eV RT-Locked high resolution spectral library to increase the confidence of identification.

Conclusions

Low energy ionization provided an additional level of information for these samples.

- Simplified chromatogram minimizing signals from hydrocarbon matrix
- Identification of co-eluting components not observed in the 70eV analysis
- High mass accuracy and precision provided confidence for statically significance interpretations
- The pellet formation process does change the volatile component composition.

References

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