Development of a GC-MS Method For the Analysis of Fermentation Derived Volatiles

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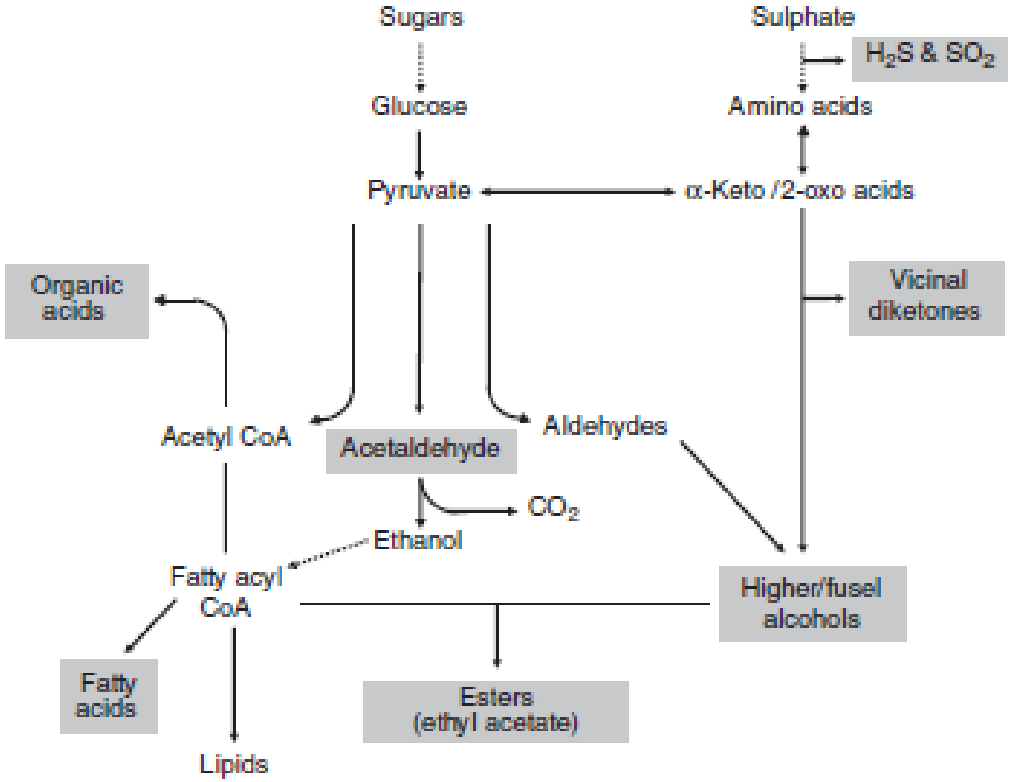
The Science of Beer

Formation of Volatile Compounds During Fermentation

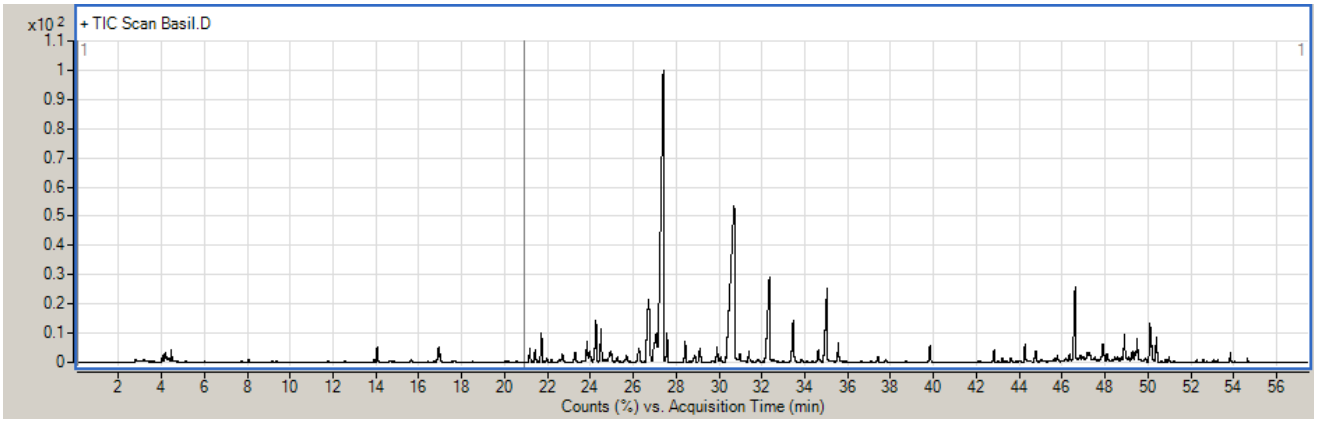


Hammond JRM (1993) Brewing yeast. *The Yeasts*, 2nd edn (Rose AH & Harrison JS, eds), pp. 5–67. Academic Press, London.

Fermentation Derived Volatiles

Compound/Class	Flavor Threshold (ppb)	Descriptor
Aldehydes		
Acetaldehyde	1000	Paint, green apple, grassy
2-Phenylacetaldehyde	100	Roses, honey
Octanal	40	Orange peel
Esters		
Ethyl Acetate	20000	Solvent
Ethyl Hexanoate	200	Sour apple, aniseed
Isoamyl Acetate	600	Bananna
2-Phenethyl Acetate	200	Roses
Alcohols		
3-Methylbutanol	40000	Alcohol, bananna
2-Phenylethanol	25000	Alcohol, rose, honey
Fatty Acid		
Octanoic Acid	13000	Goaty, wet dog
Hexanoic Acid	8000	Goaty, sweaty
Phenols		
4-Vinyl-Guaiacol	200	Cloves, smokey

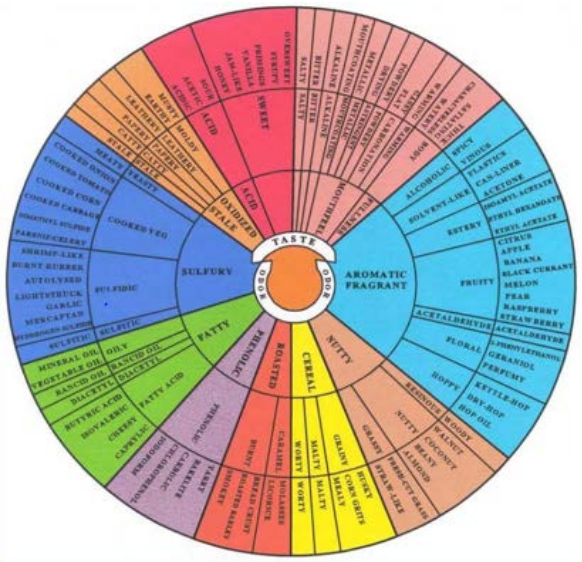
What volatile compounds are critical to control in your process?



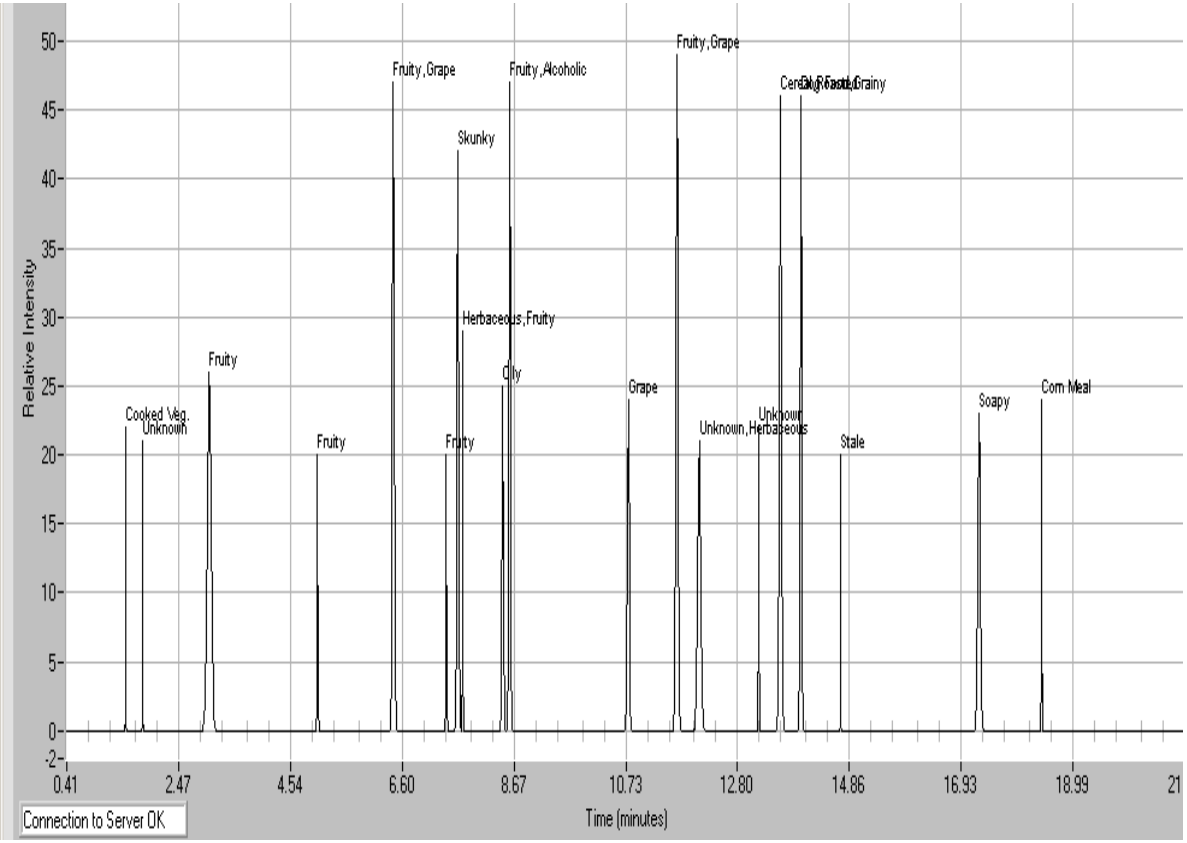
General Screening Analysis

Olfactory Analysis

Sensory – Off Flavors



Aroma Extract Dilution Analysis



- Serially dilute a sample and analyze by GC-MS with Olfactometry. The compounds that persist by olfactometry at the highest dilution levels can have the highest impact.

Fermentation Volatiles

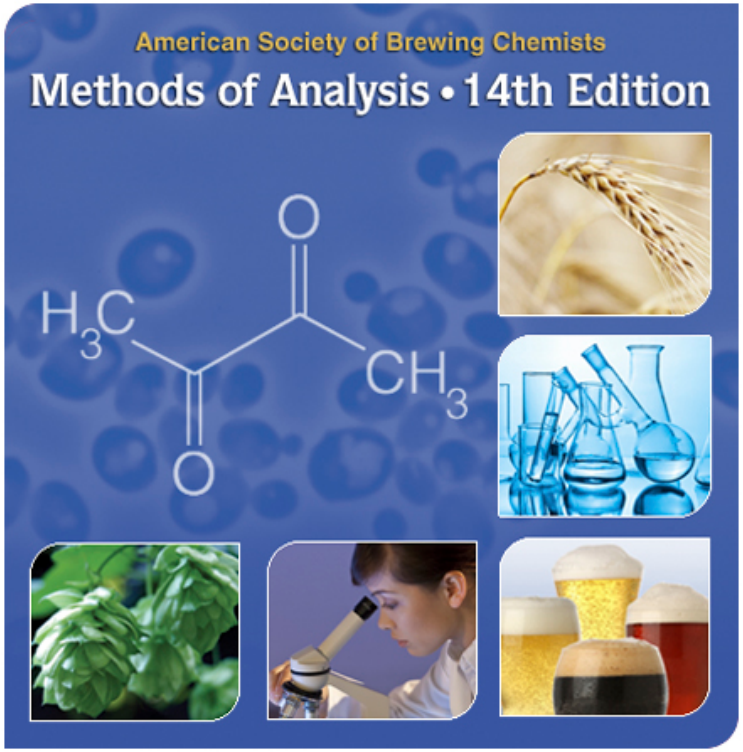
Compound/Class	Calibration Range	Flavor Threshold (ppb)	Descriptor	Notes
Acetaldehyde	0.5-15 ppm	1000	Paint, green apple, grassy	Off-Note
Ethyl Acetate	2-60 ppm	20000	Solvent	
Ethyl Hexanoate	50-2000 ppb	200	Sour apple, aniseed	
Isoamyl Acetate	0.5-15 ppm	600	Bananna	
2-Phenethyl Acetate	50-2000 ppb	200	Roses	
3-Methylbutanol	5-150 ppm	40000	Alcohol, bananna	
Octanoic Acid	50-2000 ppb	13000	Goaty, wet dog	
Ethyl Butyrate	10-300 ppb	400	Tropical Fruit	
4-Vinyl-Guaiacol	50-2000 ppb	200	Cloves, smokey	
Decanal	10-300 ppb	6	Orange	Aging Component
3-Methyl-butanal	10-300 ppb	60	Malty, cheese	Aging Component
2-Methyl-butanal	10-300 ppb	45	Green grass, cheese	Aging Component

Method Requirements

- What volatiles are critical for your beer (good and bad)?
- What are the flavor thresholds for those compounds – detection limits?
- What is the range in concentration (ppb-ppm)?
- Sample throughput (in-process and finished beer)?
- Sample preparation – time and cost?
- All analytes on 1 method?

Where to Start

- ASBC Methods
- Instrument Companies
- GC Column Suppliers
- Conferences
- Universities
- Breweries



Do your homework

❑ Method Requirements

❑ Don’t expect a turnkey solution

Use What you Have – GC-MS

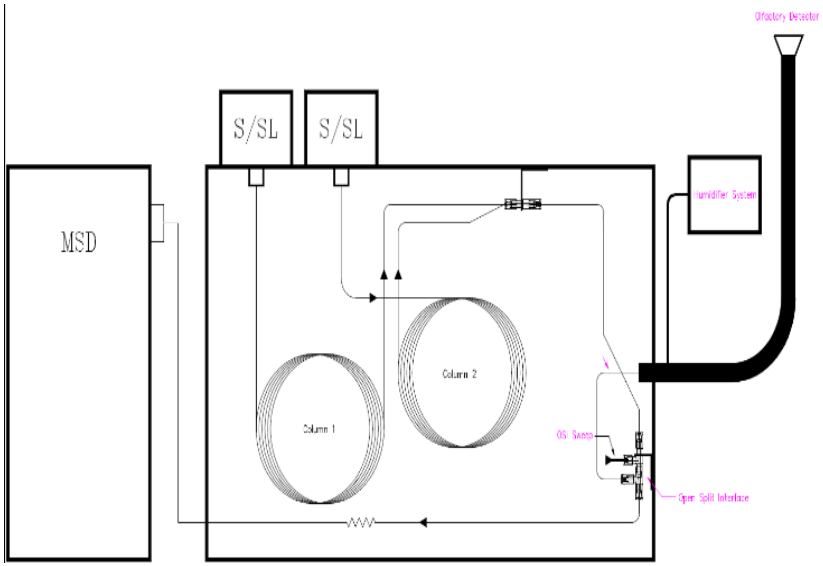
- Agilent 7890 with 5975 MS
- 2- Inlets (DB-5 or Wax Columns)
- Gerstel Autosampler with SPME and Head Space

Advantages

- Universal Detector
- Detection Limits
- Compound Identification

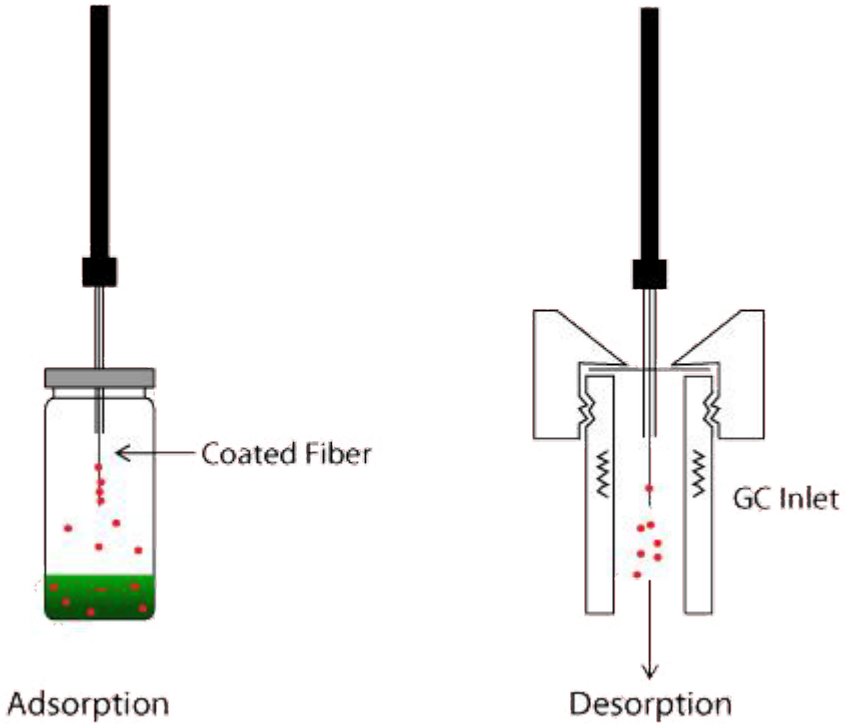
Disadvantages

- Linear Range
- Detection Limits
- Cost



Minimize Sample Prep Solid Phase Micro Extraction - SPME

- Solventless
- Automatable
- Trace Detection

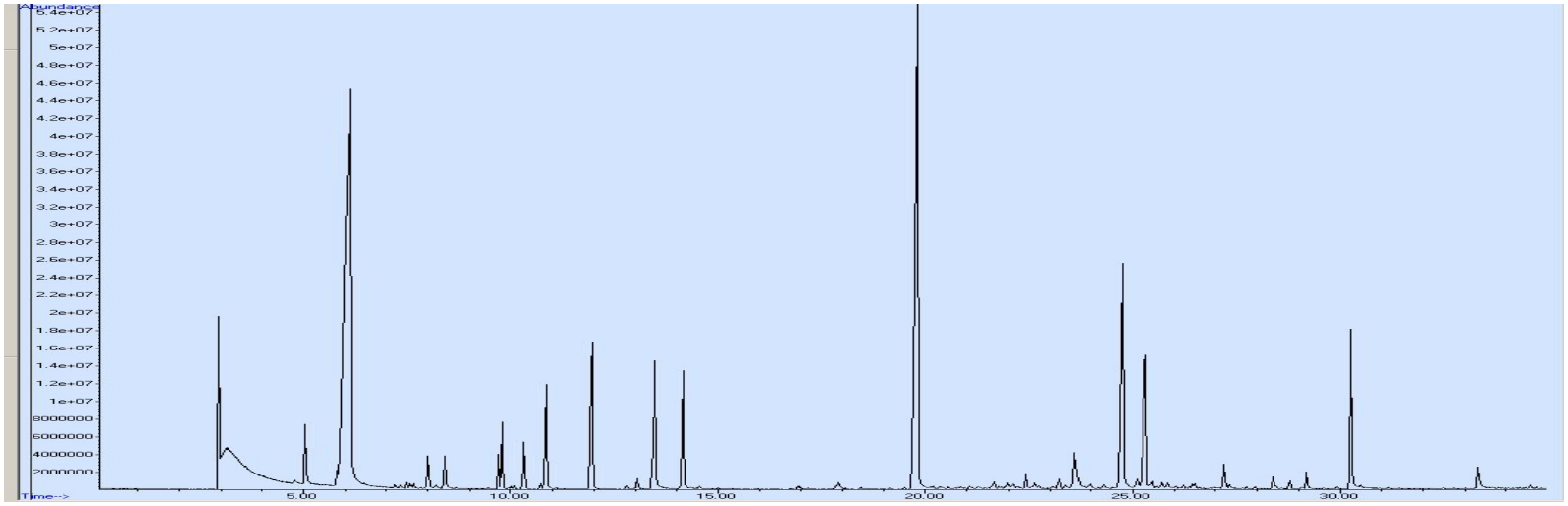


Optimized

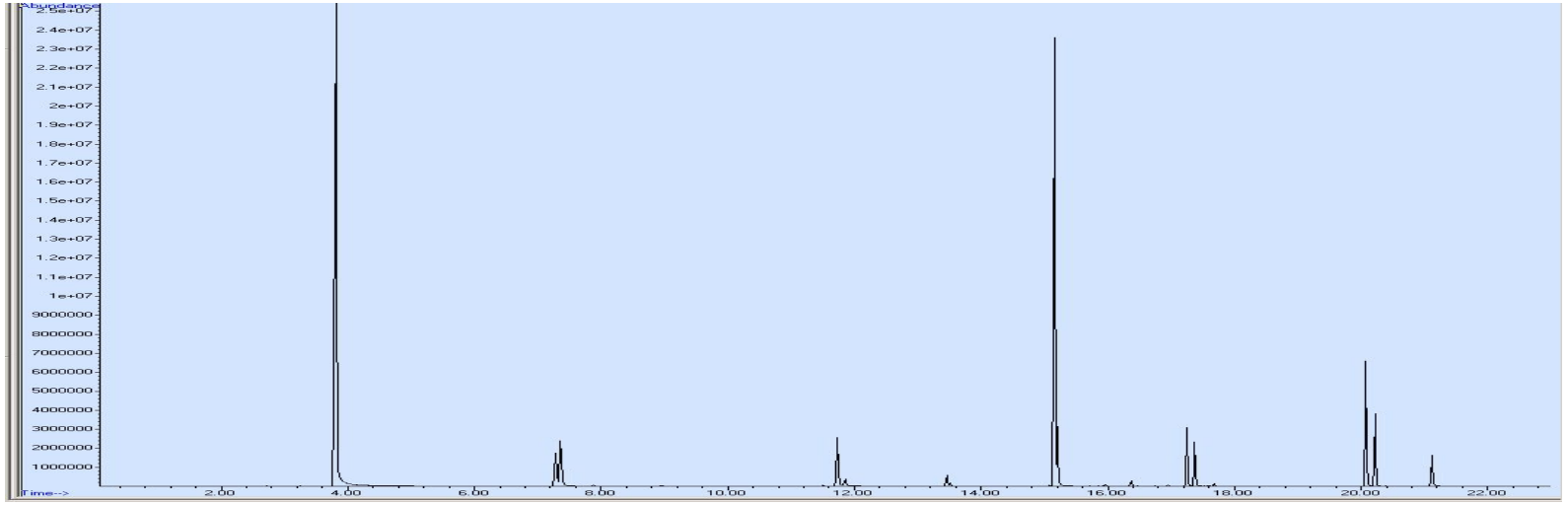
- Multiple Fiber Options
- Extraction Times
- Salting

http://2014.igem.org/Team:Valencia_UPV/Project/modules/methodology/sample_preparation

Selected Ion Monitoring



By selecting specific mass to charge ions for the analytes of interest, there is a sensitivity gain and minimal interference.



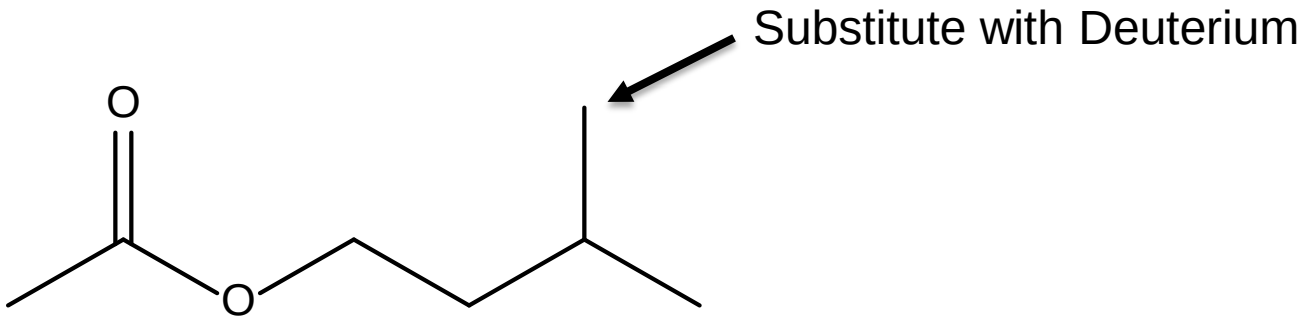
Deuterated IS

Internal Standard

- Added at a constant amount to sample.
- Used for calibration – plot the ratio of the IS to the analyte.
- Corrects for the loss of analyte during analysis.
- Similar chemical and amount, but not present in sample.

http://en.wikipedia.org/wiki/Internal_standard

Similar retention time
Differentiate with SIM



Method

- Column – DB-5MS 60m x 320 um x 1 um
- Hydrogen carrier gas
- Split 4:1
- Flow 15.75 mL/min
- Oven 35 C for 3 min, 4 C/min rise to 60, 12 C/min rise to 225 C
- Run time 23 minutes
- SIM
- Incubated at 60 C for 5 min
- Extracted for 5 min

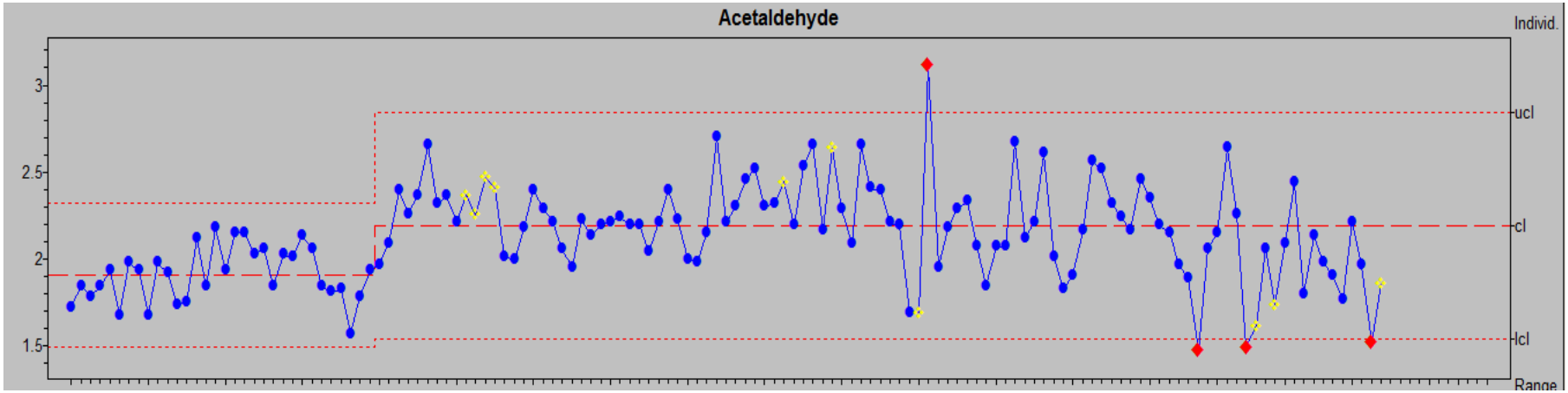
SPME Fiber - 55µm/30µm DVB/Carboxen™-PDMS, StableFlex

Validation

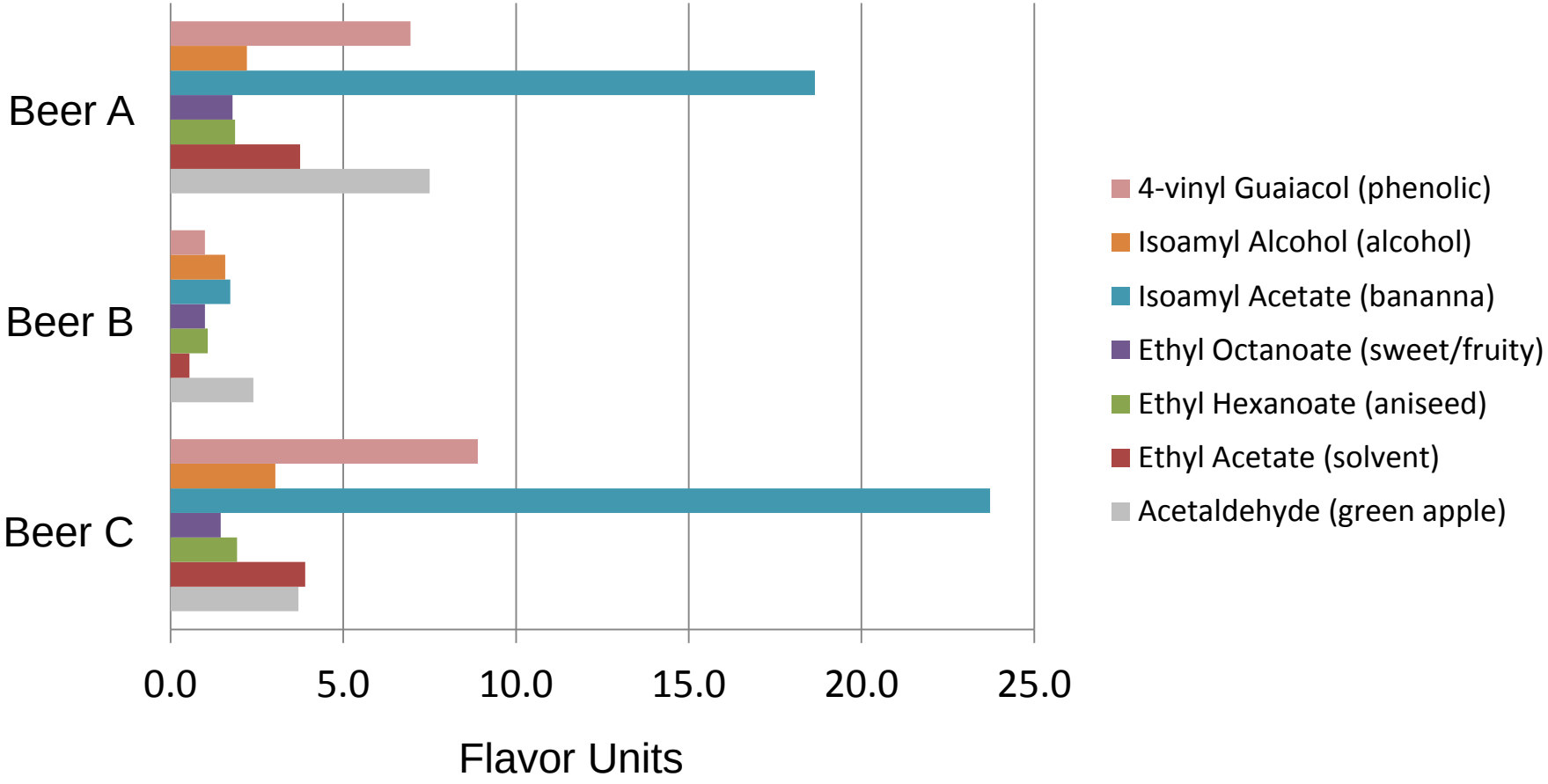
Analyte	R2 Value	Std. Concentration	Measured	Residual	LOD (ppb)	LOQ (ppb)	Recovery
Acetaldehyde	0.992	500	844.3	-344.3	257.6	772.8	75.0
Ethyl Acetate	0.998	2000	2629.7	-629.7	87.2	261.7	89.7
3-methylbutanal	0.999	10	10.4	-0.4	4.9	14.6	109.0
2-methylbutanal	0.999	10	10.9	-0.9	1.7	5.1	112.8
Isoamyl Alcohol	0.997	5000	5376.4	-376.4	439.2	1317.6	121.5
Ethyl Butyrate	0.999	10	10.7	-0.7	0.7	2.2	97.5
Isoamyl acetate	0.999	500	636.9	-136.9	28.3	85.0	96.6
Ethyl Hexanoate	0.995	50	78.6	-28.6	4.3	12.9	94.1
Ethyl Octanoate	0.988	50	97.7	-47.7	13.0	38.9	98.3
Decanal	0.975	10	20.2	-10.2	0.7	2.0	97.0
Phenethyl Acetate	0.995	50	76.8	-26.8	19.4	58.2	87.4

BAPS/Control Charting

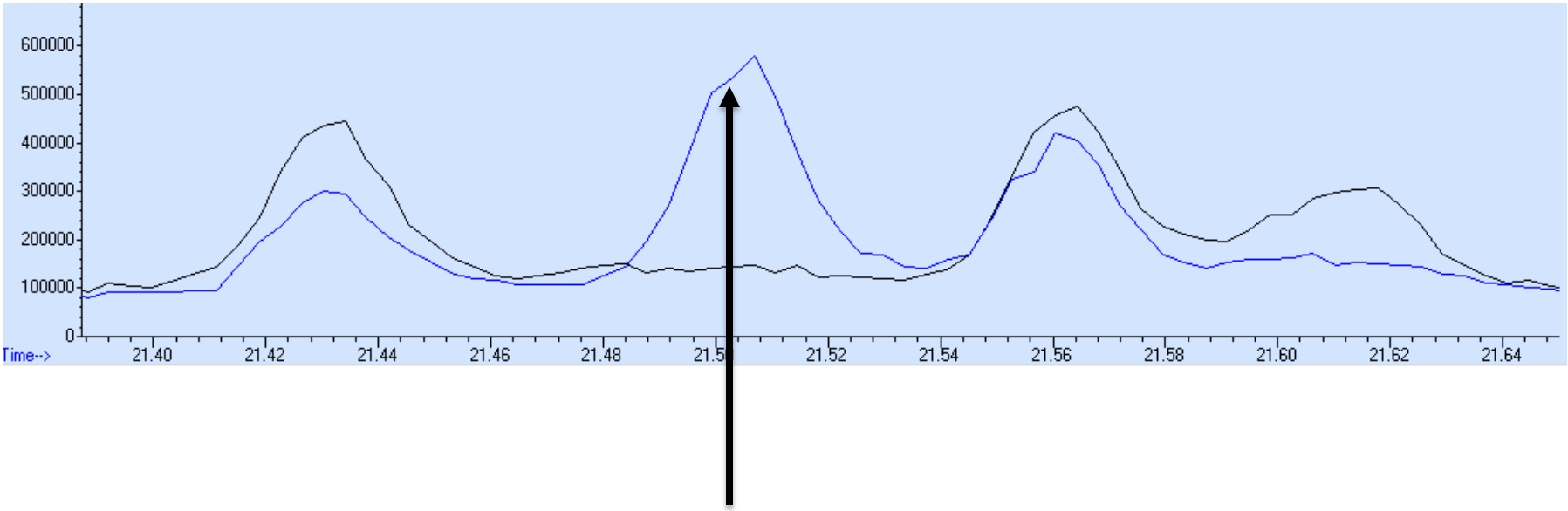
	Acetaldehyde	EA	IA alc	IAAcet	E-Hex
Baps 45	3251.0	12978.9	28583.7	762.7	141.5
Baps 45 Assigned	4470.0	12560.0	36000.0	780.0	100.0
Z-Score	-1.2	0.2	-0.1	-0.1	1.0
BAPS 47	2277.2	15219.8	32547.3	997.2	154.3
Baps 47 Assigned	2000.0	16060.0	38660.0	1230.0	100.0
Z-Score	0.3	-0.4	-0.1	-1.2	1.4



Volatile Fingerprint

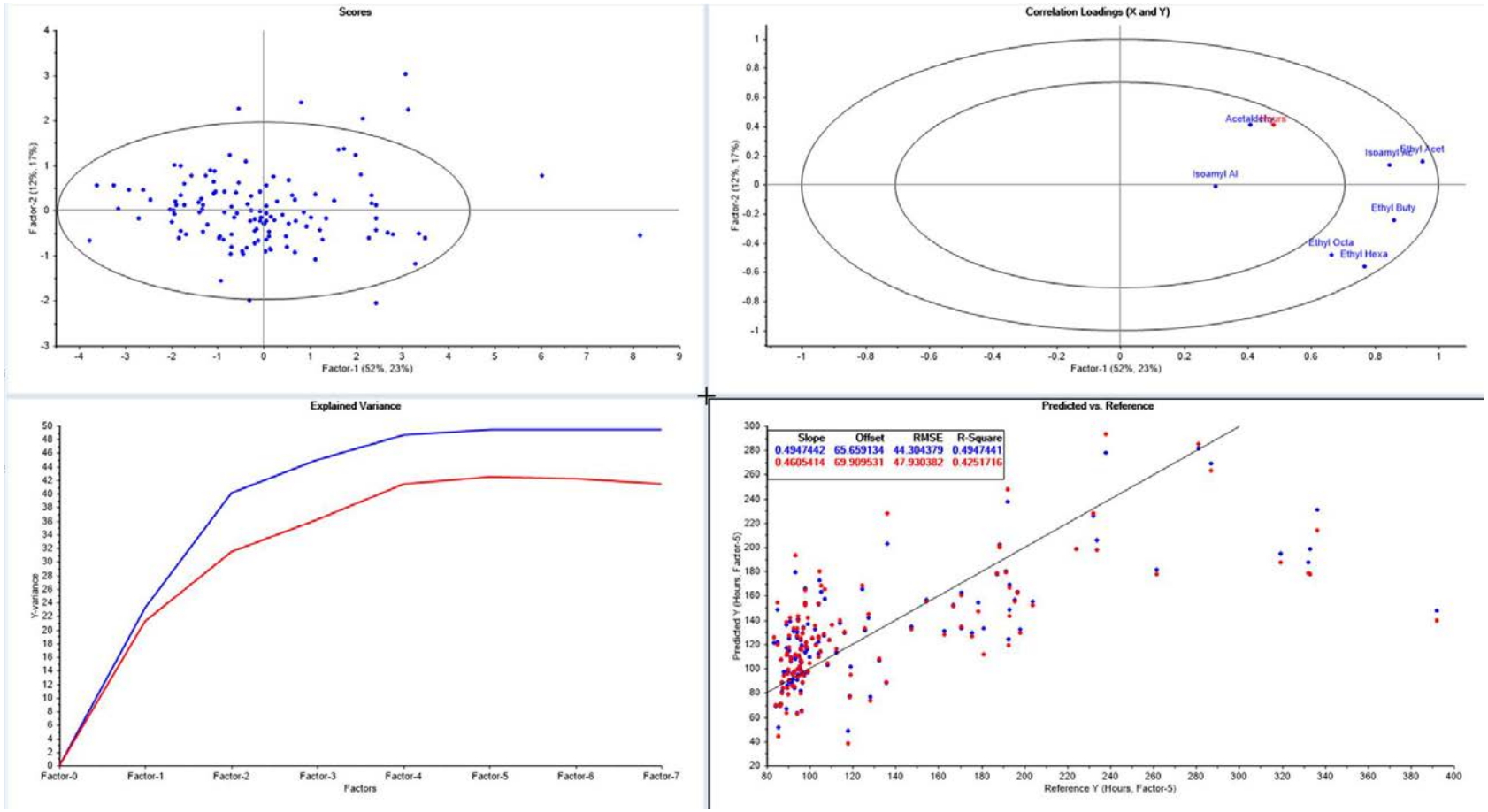


Off-Note Detection



This peak does not belong

Multivariate Analysis



- For a single brand – do fermentation hours impact the volatile profile?

Future – Aging Compounds and SAFE


2014 ASBC Annual Meeting
Instrumental methods for measuring flavor changes over the shelf life of beer
Grant Ruehle- New Belgium Brewing Co.

- Utilize Solvent Assisted Flavor Evaporation (SAFE) with AEDA to determine analytes responsible for aging aroma.
- Mine the data for trends.
- Correlate data with Sensory.

Thanks For Your Attention