Master Brewers Association of the Americas
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MBAA Annual Conference

A Novel Gas Chromatographic System to Characterize Volatile Components in Beer and its Ingredients

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PerkinElmer
For the Better

Content

- Introduction
- Chemical Analysis versus Organoleptic Perception
- System Overview
- Application Examples
- Conclusions

So, Which Country Makes the Best Beer?

- Yes – you are quite correct, it's England!

The Bungay Fleece – my local pub in England

Craft Brewing is a Growth Industry in the USA!

- **2013 Craft Beer Industry**
  - Growth of the craft brewing industry in 2013 was 18% by volume and 20% by retail dollars.
- **Overall US Beer Market in 2013**
  - Down 2%

Tasting Beer

- Besides actually making beer (of course), much of the fun is associated with drinking it!

- It's nutritious, it makes the world easier to live in and it tastes good.

- Taste is obviously subjective, but beer connoisseurs will generally consider the following when drinking a fine beer:
  - Don't drink out of the bottle
  - Don't cool the beer to Arctic temperatures
  - Use an appropriately shaped glass
  - Don't fill the glass completely
  - **These are all done to ensure that the beer aroma is involved in the tasting process (beer aroma, or 'nose' as it's called, is an important part of the formal beer-judging process)**

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Taste and Aroma

- Scientists say that 85% of flavor complexity comes from aroma
- Beer aroma and the factors affecting it are of critical interest to the brewing industry
- Traditionally, panels of experienced tasters are used to monitor the flavor of product and raw materials
  - Subjective
  - Difficult to quantify
  - Variable
  - Needs a lot of training and practice
- A more analytical approach would be a great complement to the organoleptic evaluation.

Headspace Sampling

Headspace sampling is a bit like smelling:

- Step 1 – put beer sample into a vial and seal it
- Step 2 – heat the vial for a period of time at a constant temperature
- Step 3 – extract some of the vapor and analyze it by gas chromatography
- Step 4 – quantification is possible because the concentration of each compound in the headspace is directly proportional to its concentration in the sample

Instrumentation

Analytical System for Aroma Analysis

- A headspace system – for extraction of the volatile aroma compounds
- A gas chromatograph – to perform a separation
- A mass sp – to identi compound quantify it

How Do We Correlate this analytical data with sensory perception?

Analytical System for Aroma Analysis

- A headspace system – for extraction of the volatile aroma compounds
- A gas chromatograph – to perform a separation of these compounds
- A mass spectrometer – to identify each compound and to quantify it
- **An olfactory port – for the user to experience the smell of each compound**

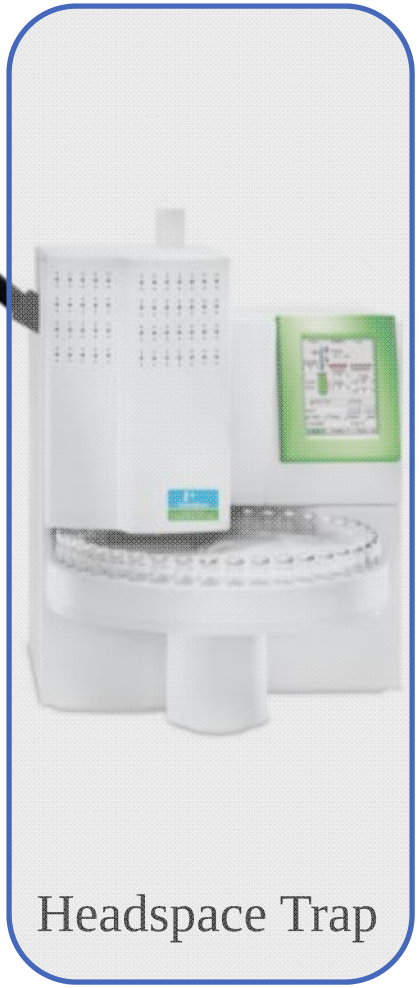
Full System

Olfactory Port Mass Spectrometer Gas Chromatograph Headspace Trap

Headspace Sampling

Olfactory Port

Mass Spectrometer Gas Chromatograph



Headspace Trap

Headspace Sampling

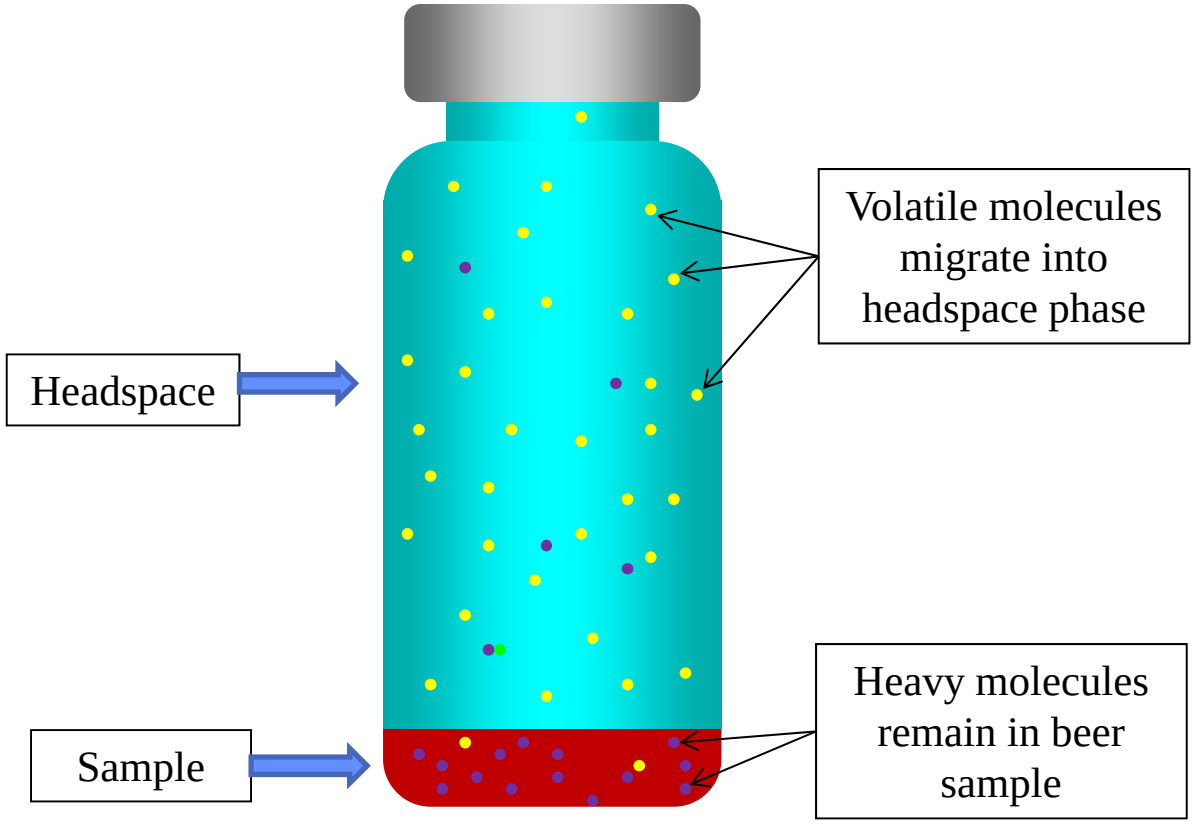
$$C_G = \frac{C_0}{(K + \beta)}$$

C_G is the concentration in the gas phase

C_0 is the concentration in the original sample

K is the partition coefficient

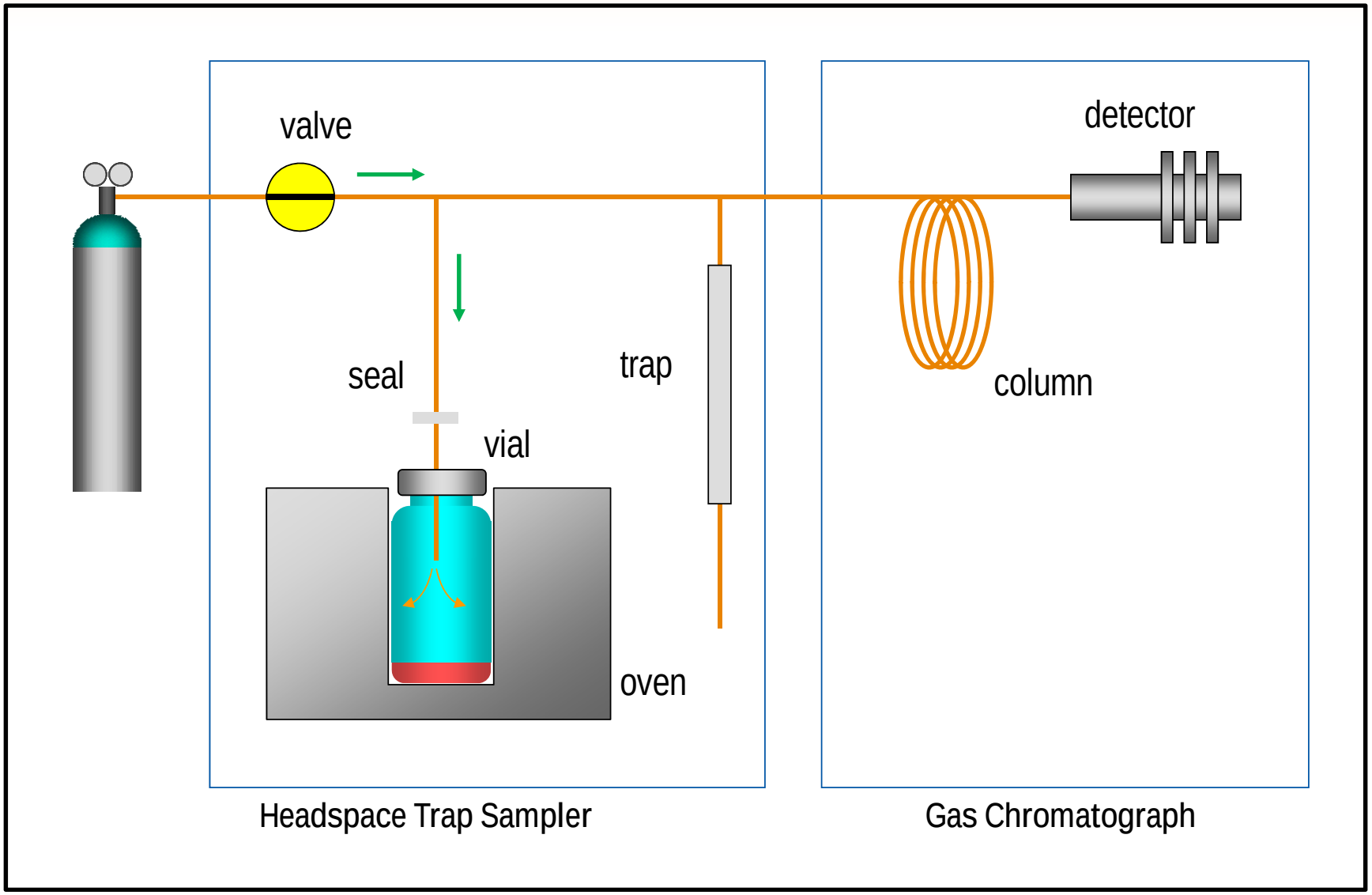
β is the phase ratio



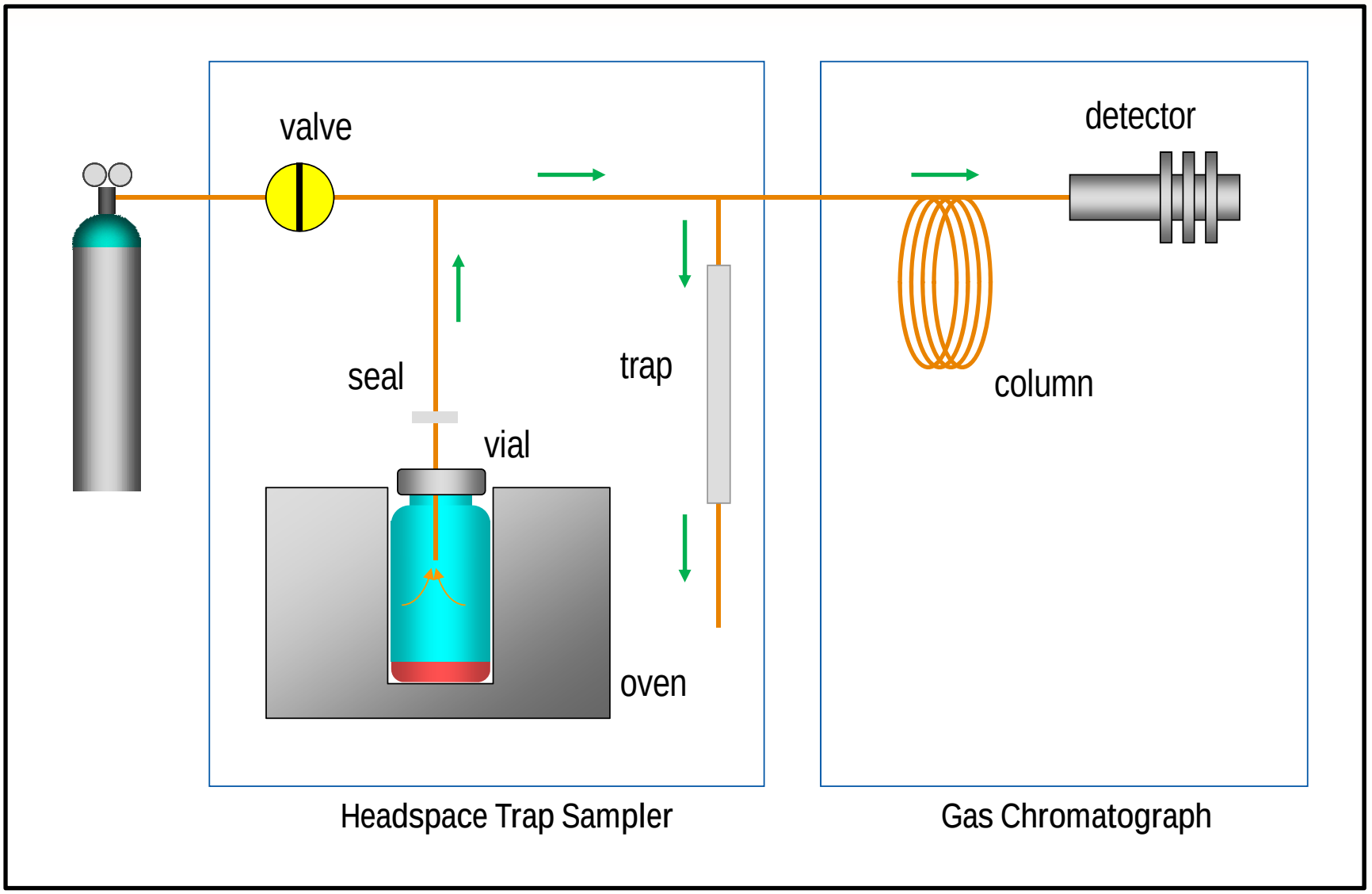
Enhanced Sensitivity with the HS Trap

- Since polar compounds in water (or beer) have very high partition coefficients – often less than 0.5% of the compound in the sample may pass into the headspace.
- With headspace without the trap, only a small fraction of the total headspace vapor will enter the column
- The headspace trap technique can enhance detection limits by 100 times by withdrawing the entire HS volume and enabling several injections from same vial to be focused on trap

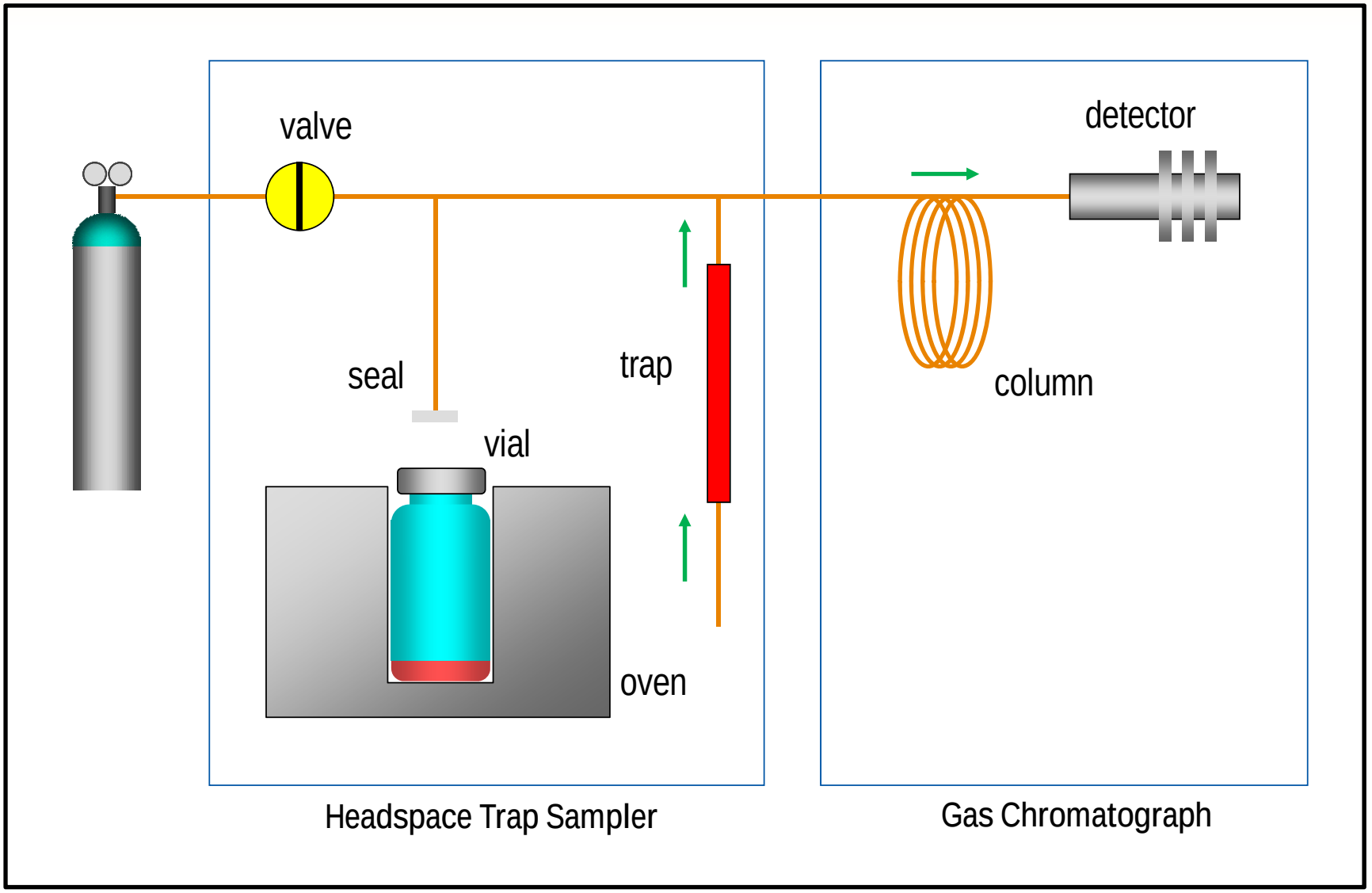
Pressurizing Equilibrated Vial



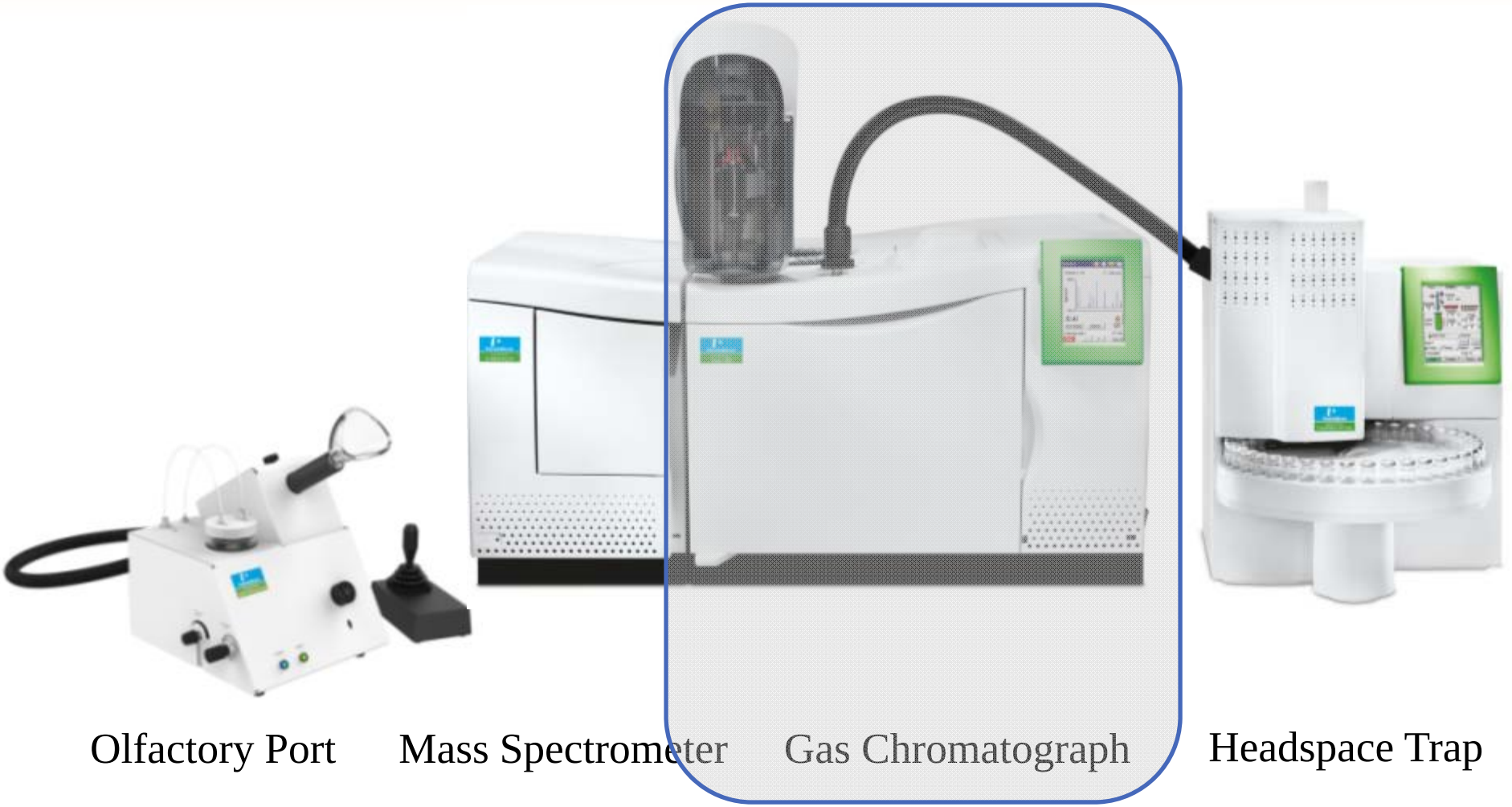
Trap Load


valve
detector
seal
trap
column
vial
oven
Headspace Trap Sampler
Gas Chromatograph

Trap Desorption onto GC Column

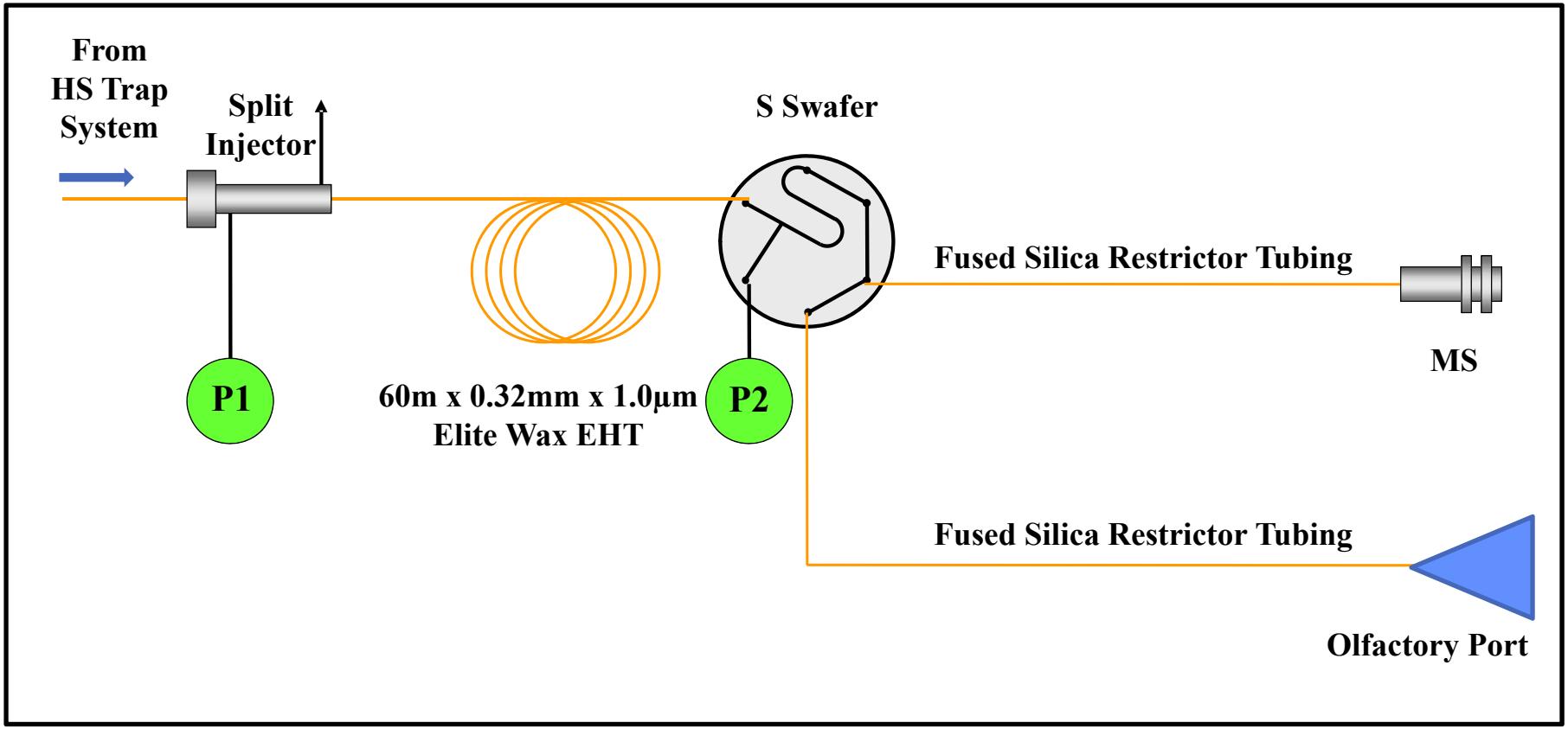


Gas Chromatography

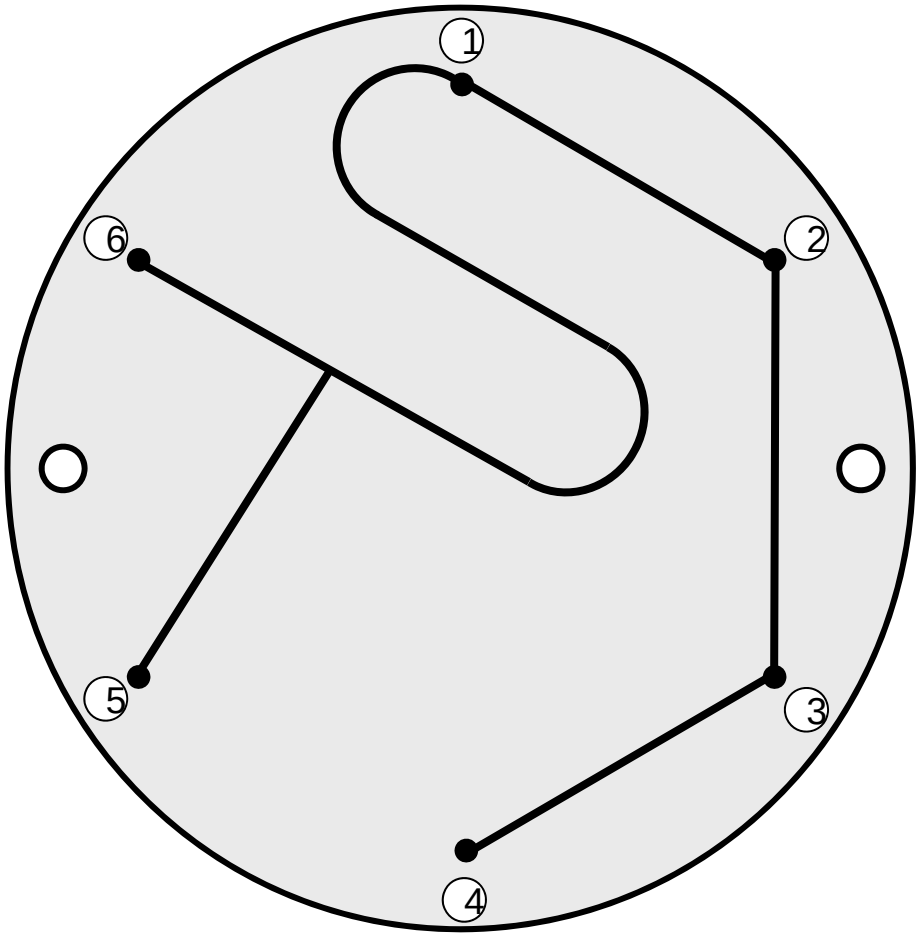


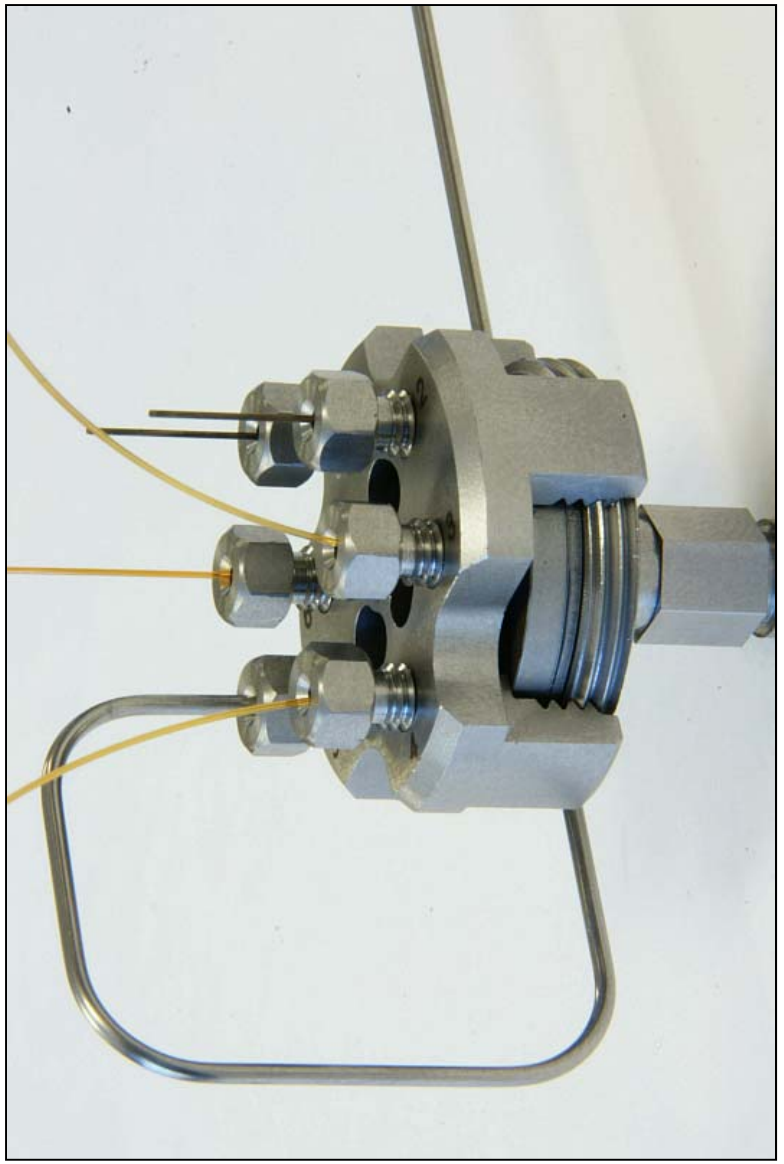
Olfactory Port Mass Spectrometer Gas Chromatograph Headspace Trap

The GC System

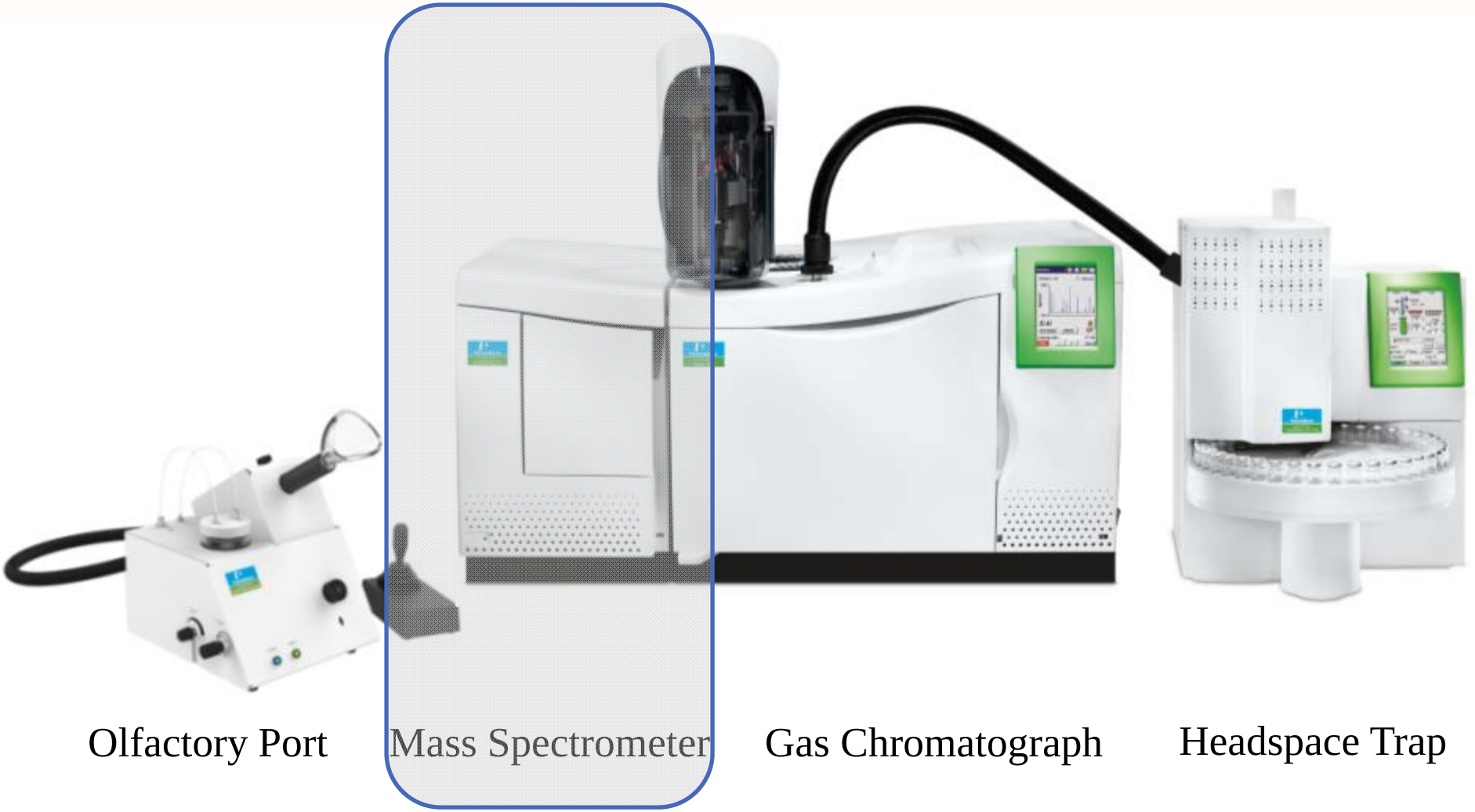


The S Swafer



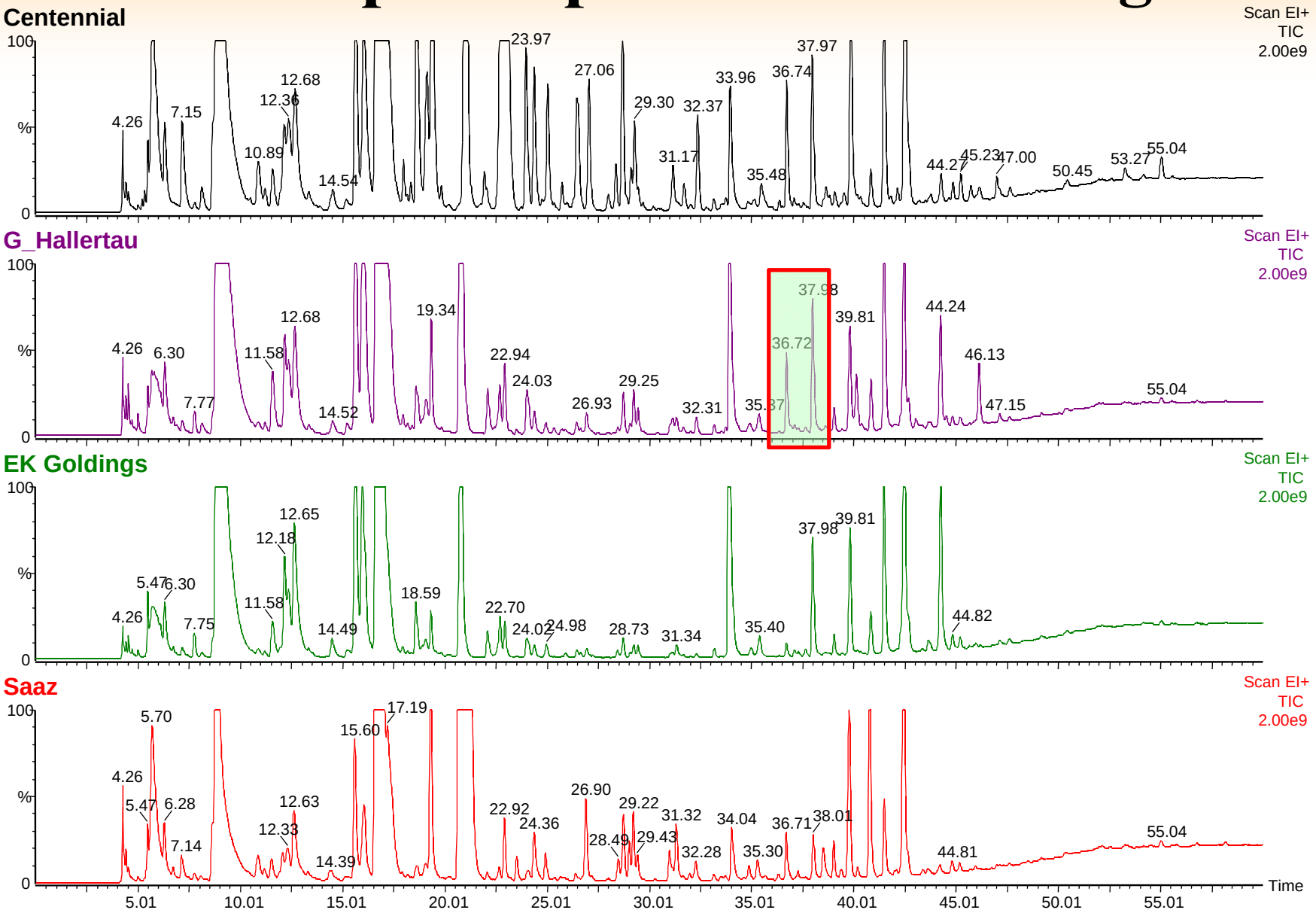


Mass Spectrometry

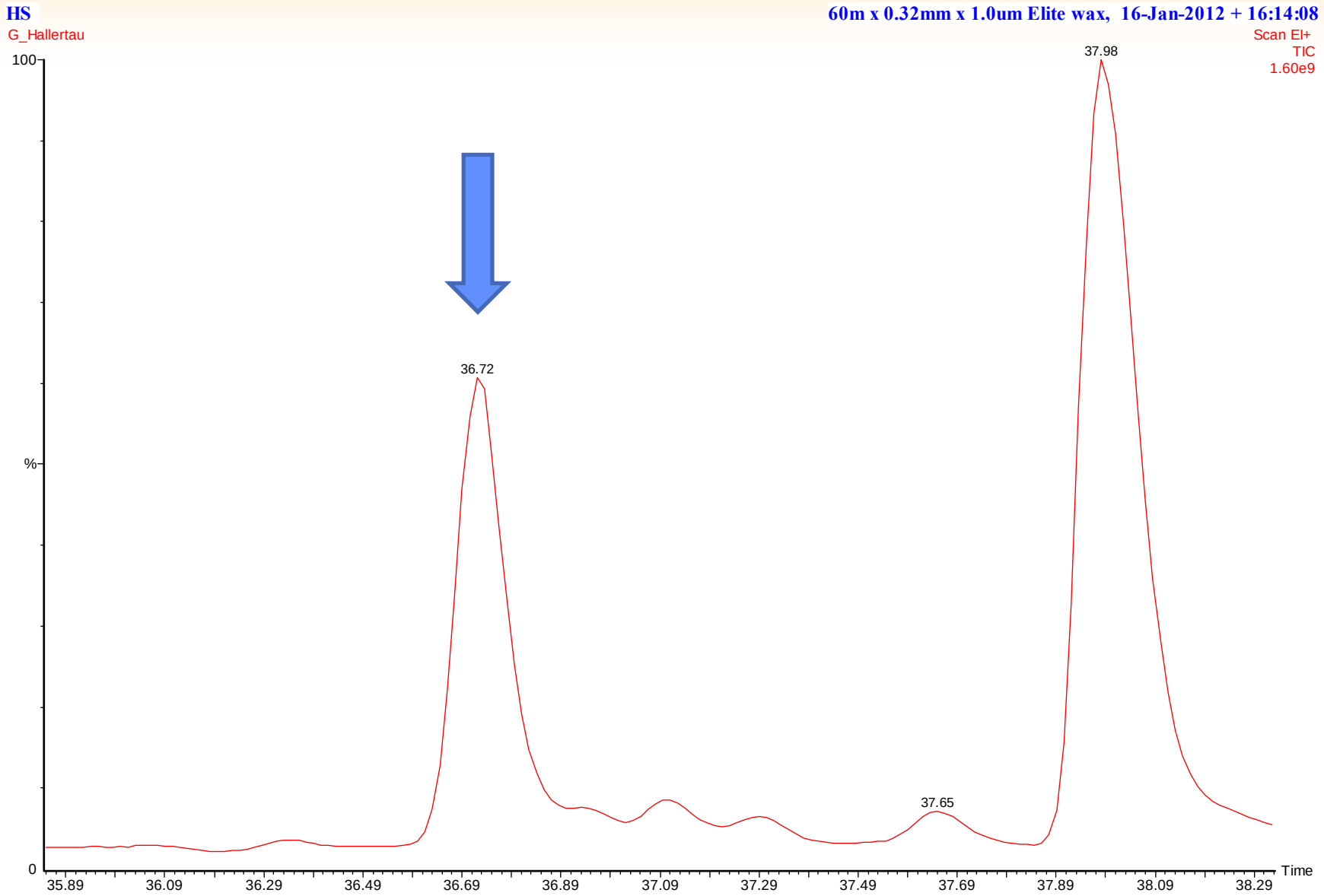


Olfactory Port | Mass Spectrometer | Gas Chromatograph | Headspace Trap

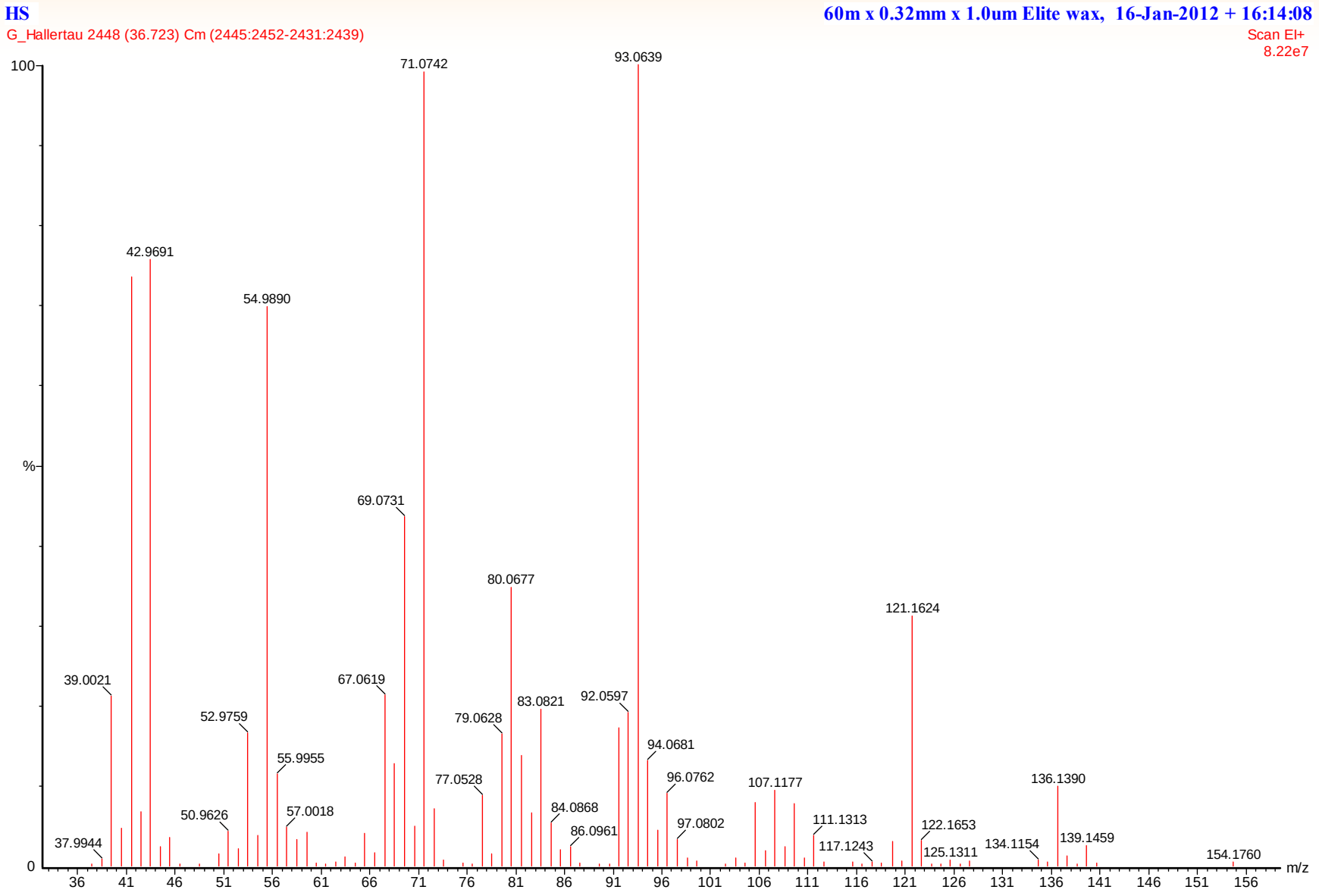
Some Example Hop Mass Chromatograms



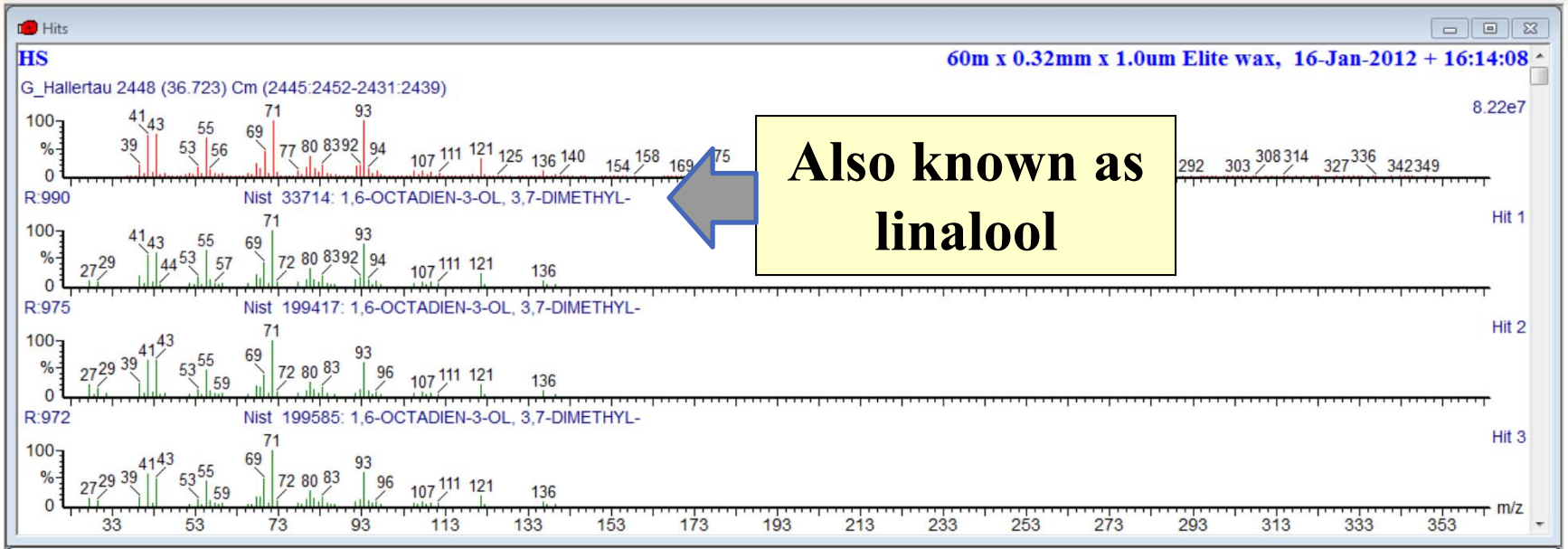
Detail from Hallertau Hop Chromatogram



Mass spectrum from peak at 36.72 minutes



Library Search



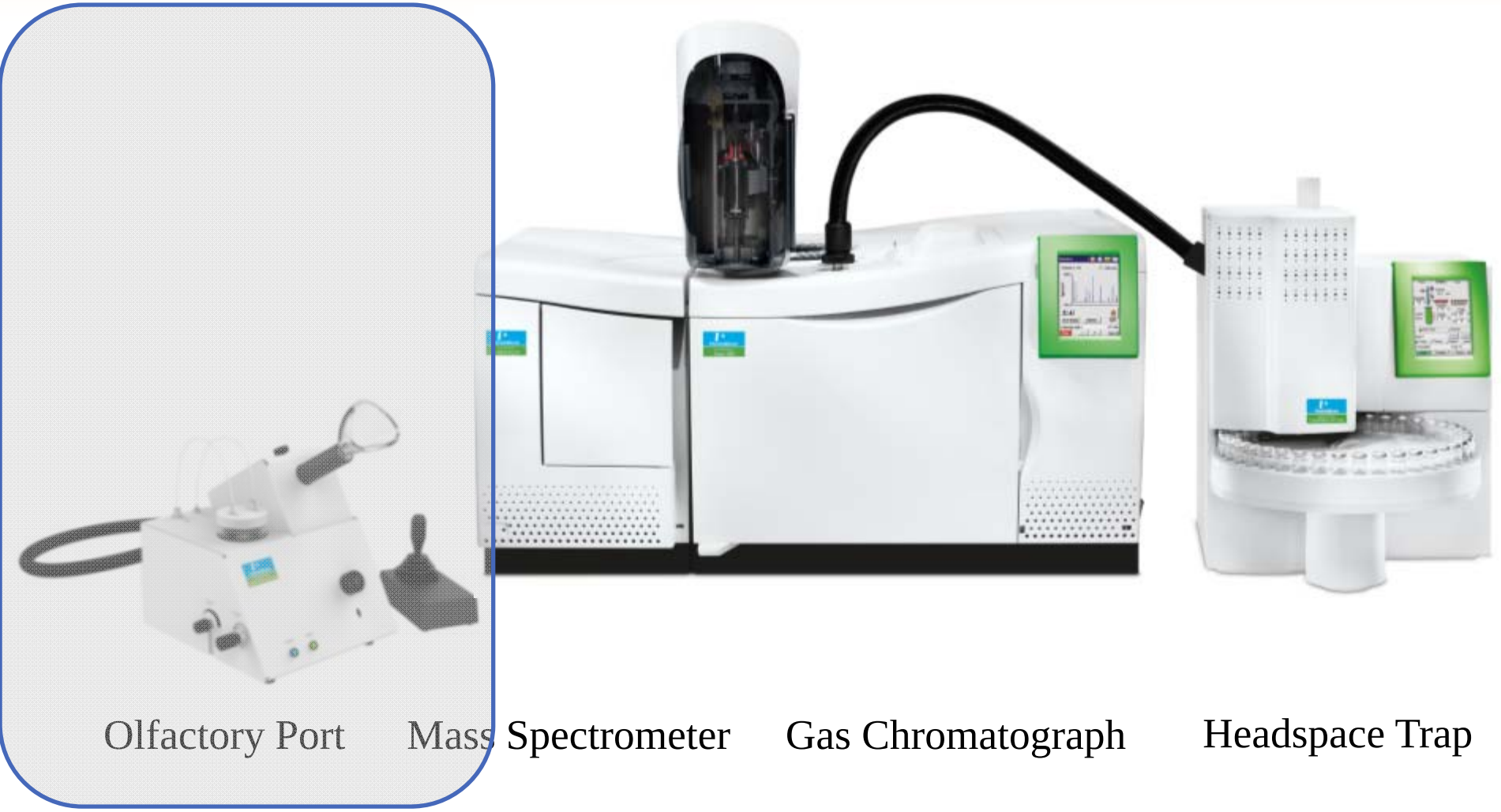
Also known as linalool

Hit List

HS — 1g German Hallertau leaves (vac) (old)

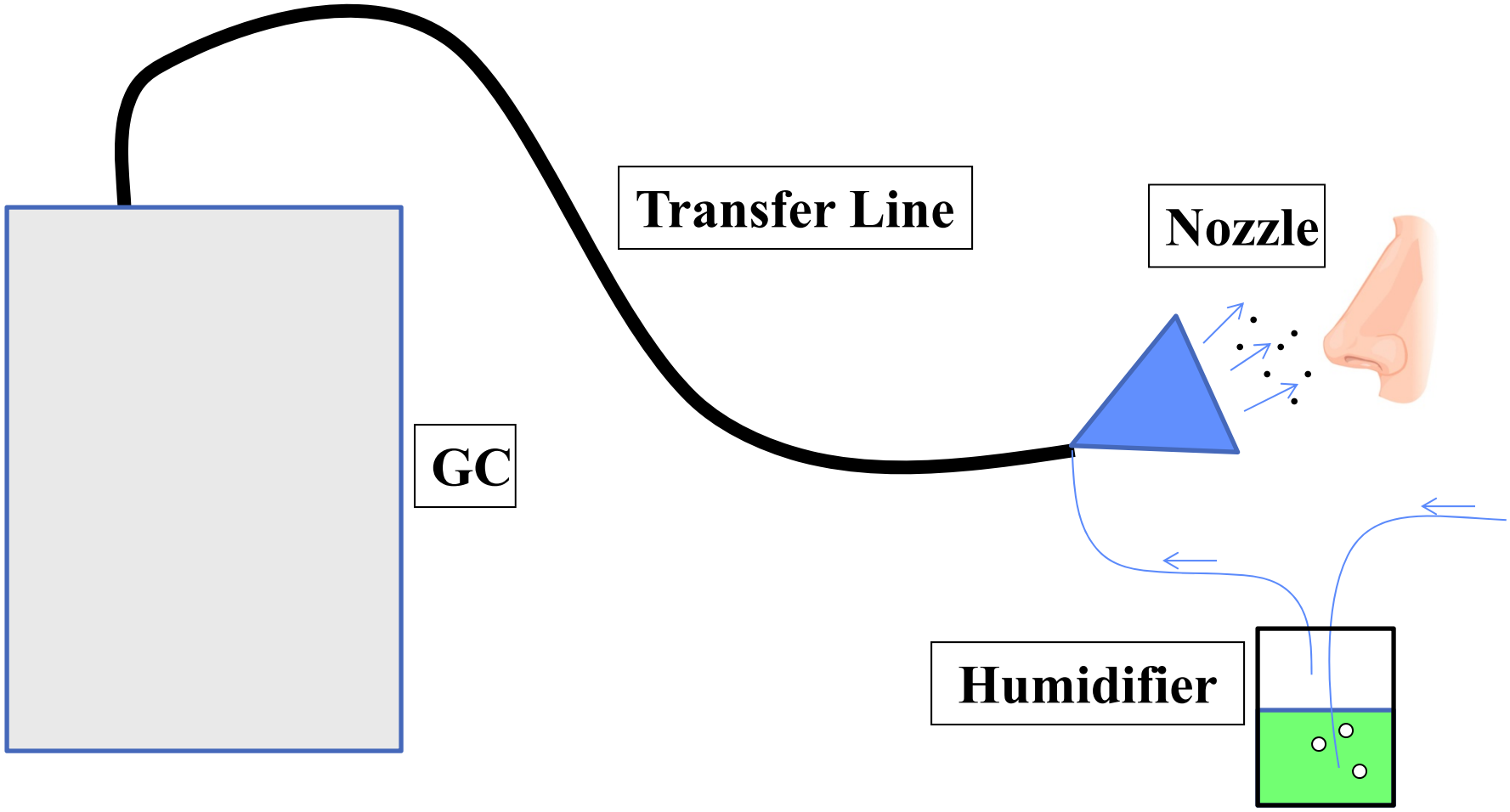
Hit	REV	for	Compound Name	M.W.	Formula	CAS	Library
1	990	986	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	154	C10H18O	78-70-6	Nist
2	975	967	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	154	C10H18O	78-70-6	Nist
3	972	960	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	154	C10H18O	78-70-6	Nist
4	930	888	1,5-DIMETHYL-1-VINYL-4-HEXENYL BUTYRATE	224	C14H24O2	78-36-4	Nist
5	919	873	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-, ACETATE	196	C12H20O2	115-95-7	Nist
6	918	880	1,5-DIMETHYL-1-VINYL-4-HEXENYL BUTYRATE	224	C14H24O2	78-36-4	Nist
7	918	877	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-, 2-AMINOBENZOATE	273	C17H23O2N	7149-26-0	Nist
8	888	845	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-, ACETATE	196	C12H20O2	115-95-7	Nist
9	887	835	LINALYL ISOBUTYRATE	224	C14H24O2	78-35-3	Nist
10	884	841	LINALYL ISOBUTYRATE	224	C14H24O2	78-35-3	Nist
11	882	848	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-, FORMATE	182	C11H18O2	115-99-1	Nist
12	873	837	TERPINEOL, CIS-.BETA.-	154	C10H18O	7299-41-4	Nist
13	862	817	BICYCLO[3.1.0]HEXAN-2-OL, 2-METHYL-5-(1-METHYLETHYL)-, (1.ALPHA.,2.BET	154	C10H18O	15537-55-0	Nist
14	846	816	CYCLOHEXANOL, 1-METHYL-4-(1-METHYLETHENYL)-	154	C10H18O	138-87-4	Nist
15	843	816	CYCLOHEXANOL, 1-METHYL-4-(1-METHYLETHENYL)-	154	C10H18O	138-87-4	Nist
16	841	747	1,6-NONADIEN-3-OL, 3,7-DIMETHYL-	168	C11H20O	10339-55-6	Nist
17	840	791	1,5-DIMETHYL-1-VINYL-4-HEXENYL BUTYRATE	224	C14H24O2	78-36-4	Nist
18	838	794	1,2-DIHYDROPYRIDINE, 1-(1-OXOBUTYL)-	151	C9H13ON	900132-46-2	Nist
19	830	776	CIS-.BETA.-TERPINEOL	154	C10H18O	7299-40-3	Nist
20	828	733	1,6,10-DODECATRIEN-3-OL, 3,7,11-TRIMETHYL-	222	C15H26O	7212-44-4	Nist

Olfactometry



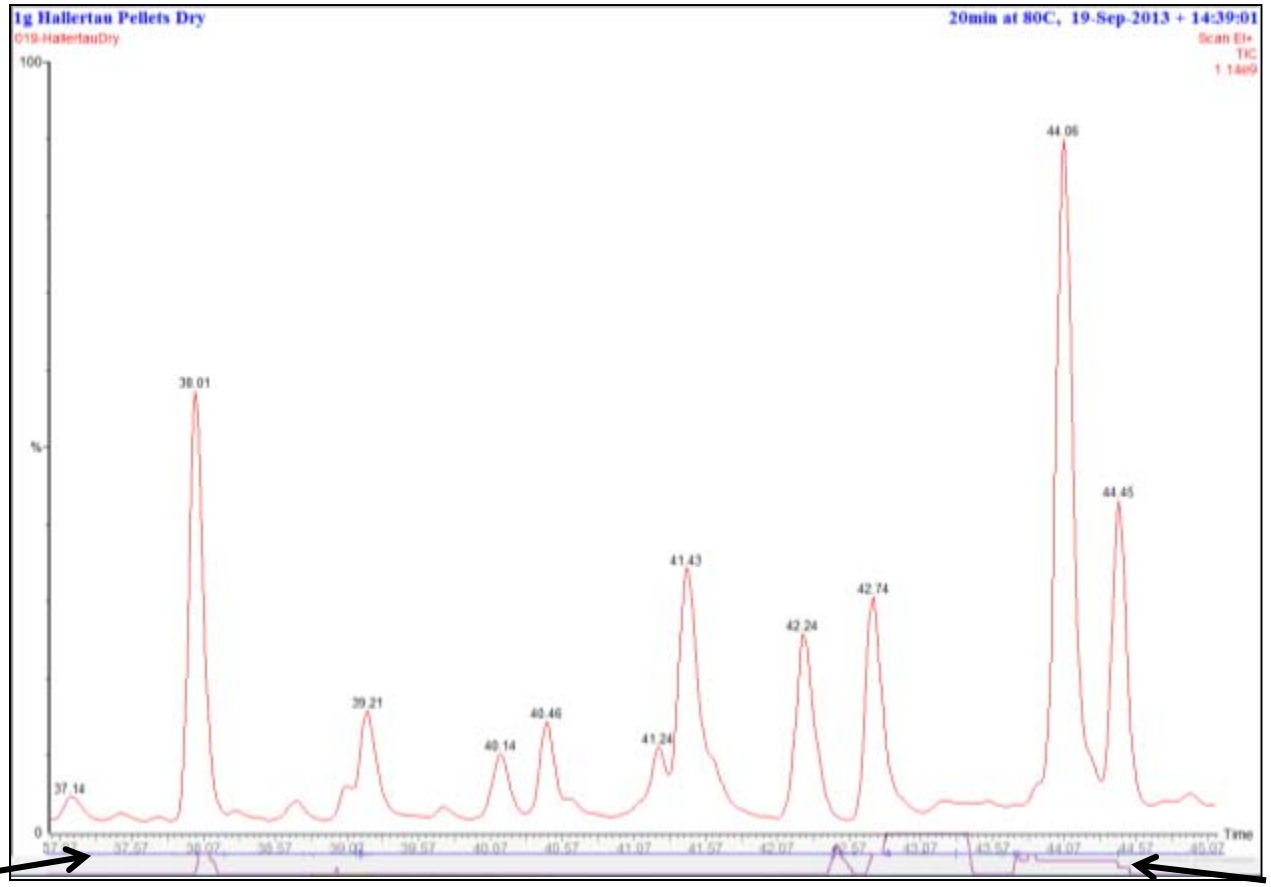
Olfactory Port Mass Spectrometer Gas Chromatograph Headspace Trap

Olfactory Port



The SNFR Olfactory Port

Audio and Intensity Activity is Overlaid on Chromatography



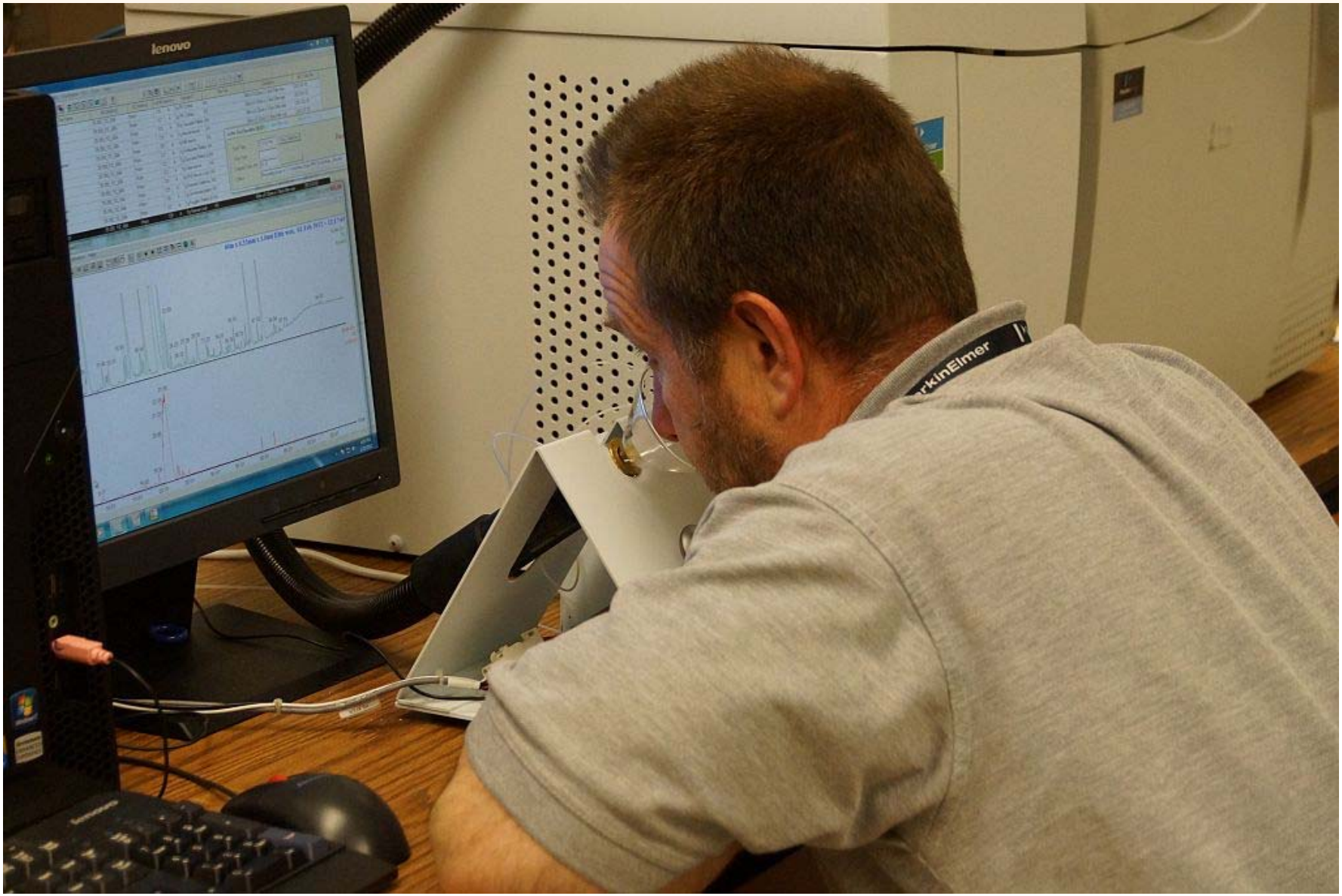
audio stream

intensity stream

Example of Report File

Project Name OKTOBERFEST.PRO		
Sample Name 019-HallertauDry		
Start Time 9/19/2013 2:39:02 PM		
Duration 60.00		
Time Stamp	Spoken Text	Intensity
1.05	Coming up on a minute	0
2.13	two minutes	0
5.15	a sweet smell	0
5.20	very faint	0
6.07	nothing there	0
6.65	very very faint smell	2
6.88	off order	3
7.12	like sour milk	2
7.25	sour milk	4
7.30	was a very good banana smell	5
7.35	fruity smell	4
8.18	like a sour milk	4
8.23	sour milk	4
9.17	fruit there	2
10.02	nothing there	0
10.10	large peak and I smell nothing	0
11.52	burning smell	2
11.58	Almost woody	0
12.00	little sweet	1
12.45	almost a hint of coffee	0
13.22	that’s an off smell	3
13.25	a rancid smell	3
13.82	something	3
13.88	almost	0
13.90	medical	0
15.43	medical smell	2
15.47	is almost toffee like	2
15.57	very pleasing	4
16.43	off order	0
17.92	slight sweet	0
18.58	bubblegum	0
19.88	hint of something sweet	0
21.00	off order of skunk	3
21.08	definite skunk	5
22.90	something	3
23.02	almost like a match	1
23.07	a sulfur smell	0
25.18	subtle	2
25.22	subtle	0
25.33	not quite sure what that was	0
25.70	nothing there	0
30.70	little off odor	1
33.67	foul smell	2
36.23	smell of cardboard must	0
36.35	bananas	2
36.82	almost mint	2
38.08	That was a nice fruit	3
38.20	very citrus	0
42.47	hot	4
42.50	pepper	2
42.70	again	3
42.82	it's an off odor	6
42.85	are very bad off order	6
43.08	a sweaty socks smell	6
43.72	that's a fruity smell	2
43.73	very pleasing	2
45.78	floral	2

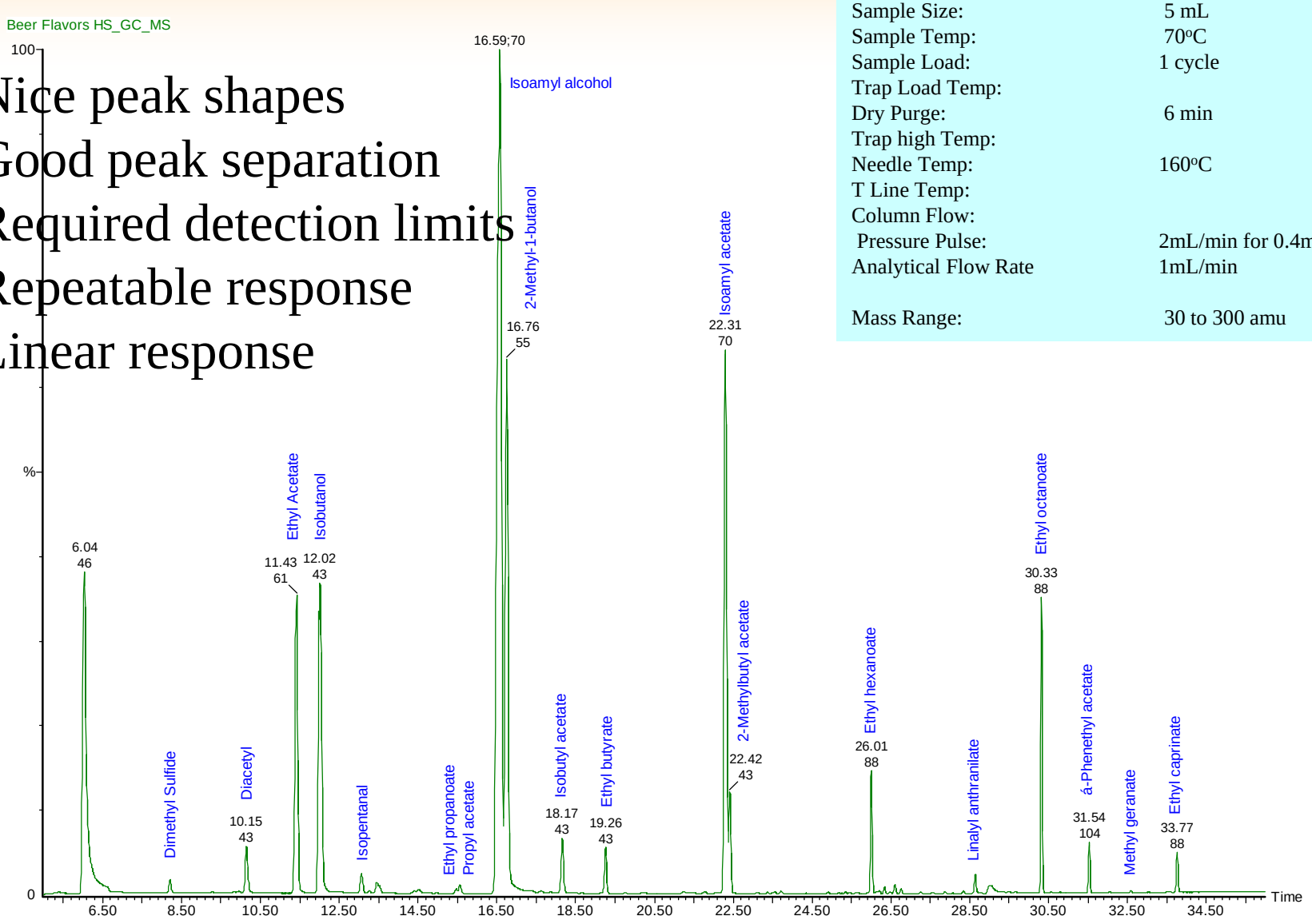
Mark Szamatulski Using Prototype System



Applications

Beer - Component Identification by MS

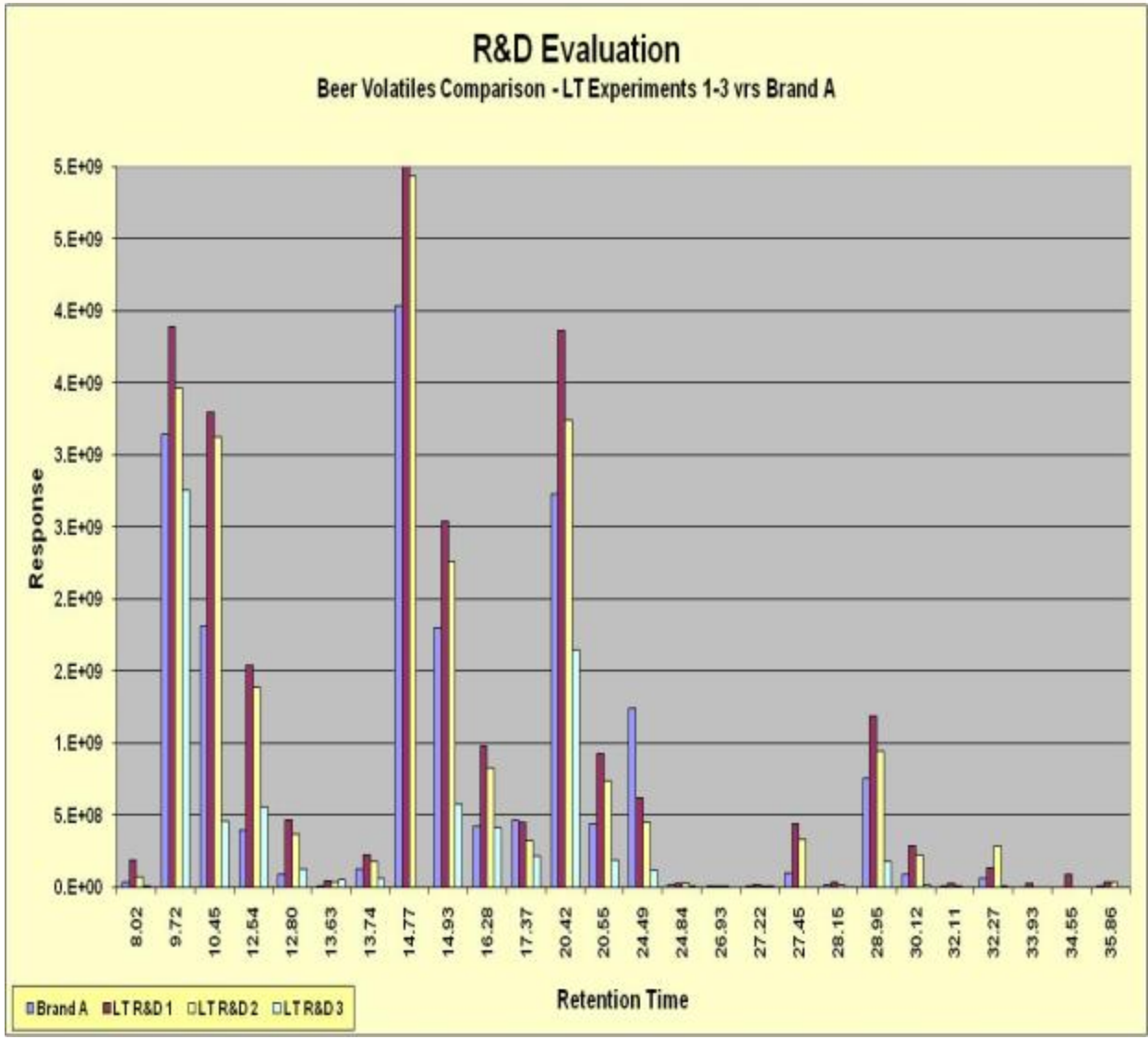
- Nice peak shapes
- Good peak separation
- Required detection limits
- Repeatable response
- Linear response



Sample Size:	5 mL
Sample Temp:	70°C
Sample Load:	1 cycle
Trap Load Temp:	25°C
Dry Purge:	6 min
Trap high Temp:	300°C
Needle Temp:	160°C
T Line Temp:	180°C
Column Flow:	
Pressure Pulse:	2mL/min for 0.4min
Analytical Flow Rate	1mL/min
Mass Range:	30 to 300 amu

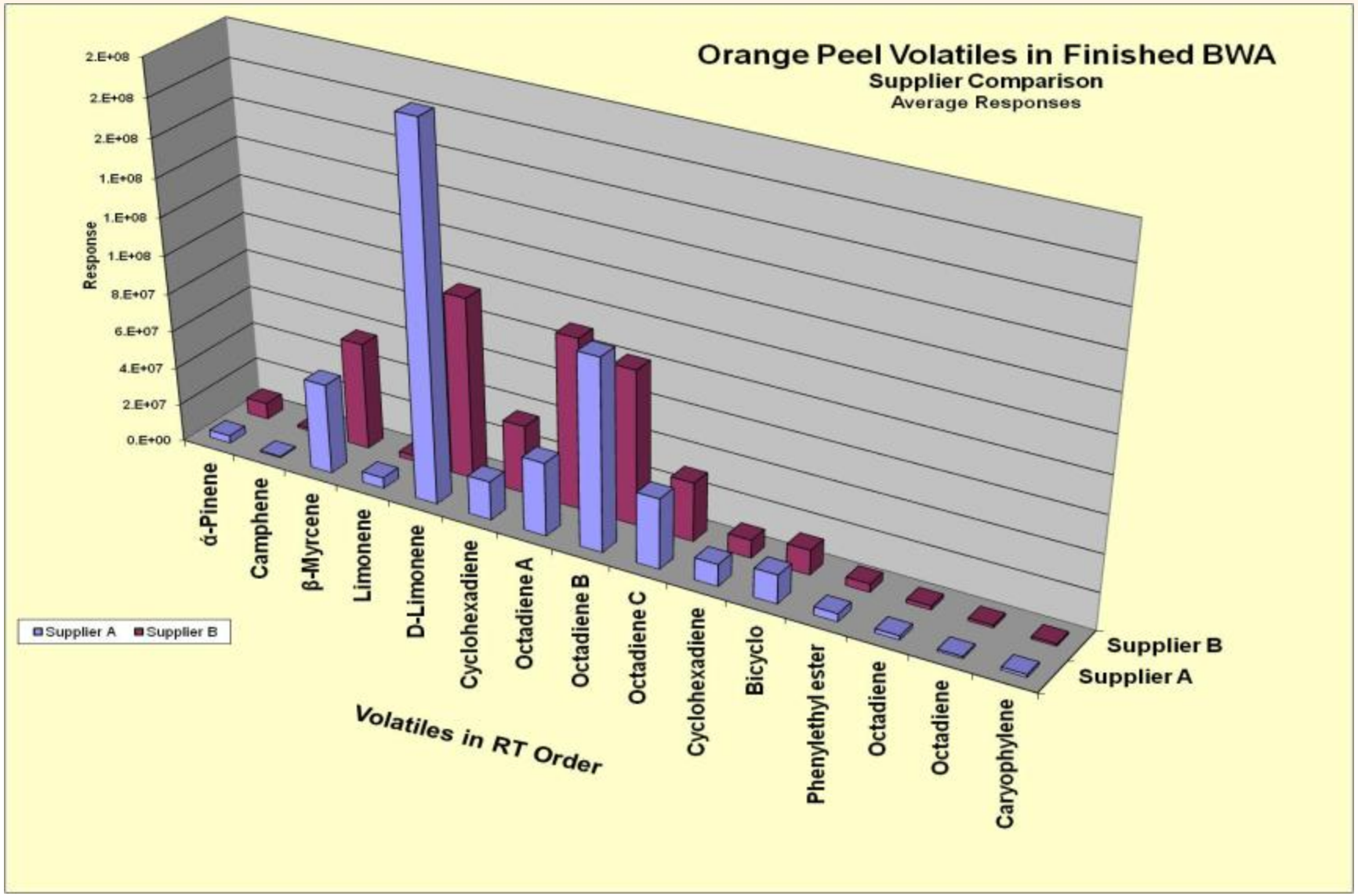
Beer Volatile Comparison

Compound Name	Retention Time
1-Propanol	8.02
2-Butanone, 4-hydroxy	9.72
1-Propanol, 2-methyl	10.45
1-Butanol, 3-methyl	12.54
1-Butanol, 2-methyl	12.80
Propanoic acid ethyl ester	13.63
n-Propyl acetate	13.74
Mixture of methyl butanols	14.77
Mixture of methyl butanols	14.93
Acetic acid, 2-methylpropyl ester	16.28
Butanoic acid, ethyl ester	17.37
1-Butanol, 3-methyl-, acetate	20.42
1-Butanol, 2-methyl-, acetate	20.55
Hexanoic acid, ethyl ester	24.49
Acetic acid hexyl ester	24.84
Heptanoic acid, ethyl ester	26.93
Acetic acid, heptyl ester	27.22
Phenyl ethyl alcohol	27.45
Octanoic acid	28.15
Octanoic acid, ethyl ester	28.95
Acetic acid, 2-phenylethyl ester	30.12
Ethyl9-decanoate	32.11
Decanoic acid ethyl ester	32.27
Caryophyllene	33.93
Alpha caryophyllene	34.55
Decanoic acid ethyl ester	35.86



Data Courtesy of Long Trail Brewery, VT

Adjunct Supplier Comparison

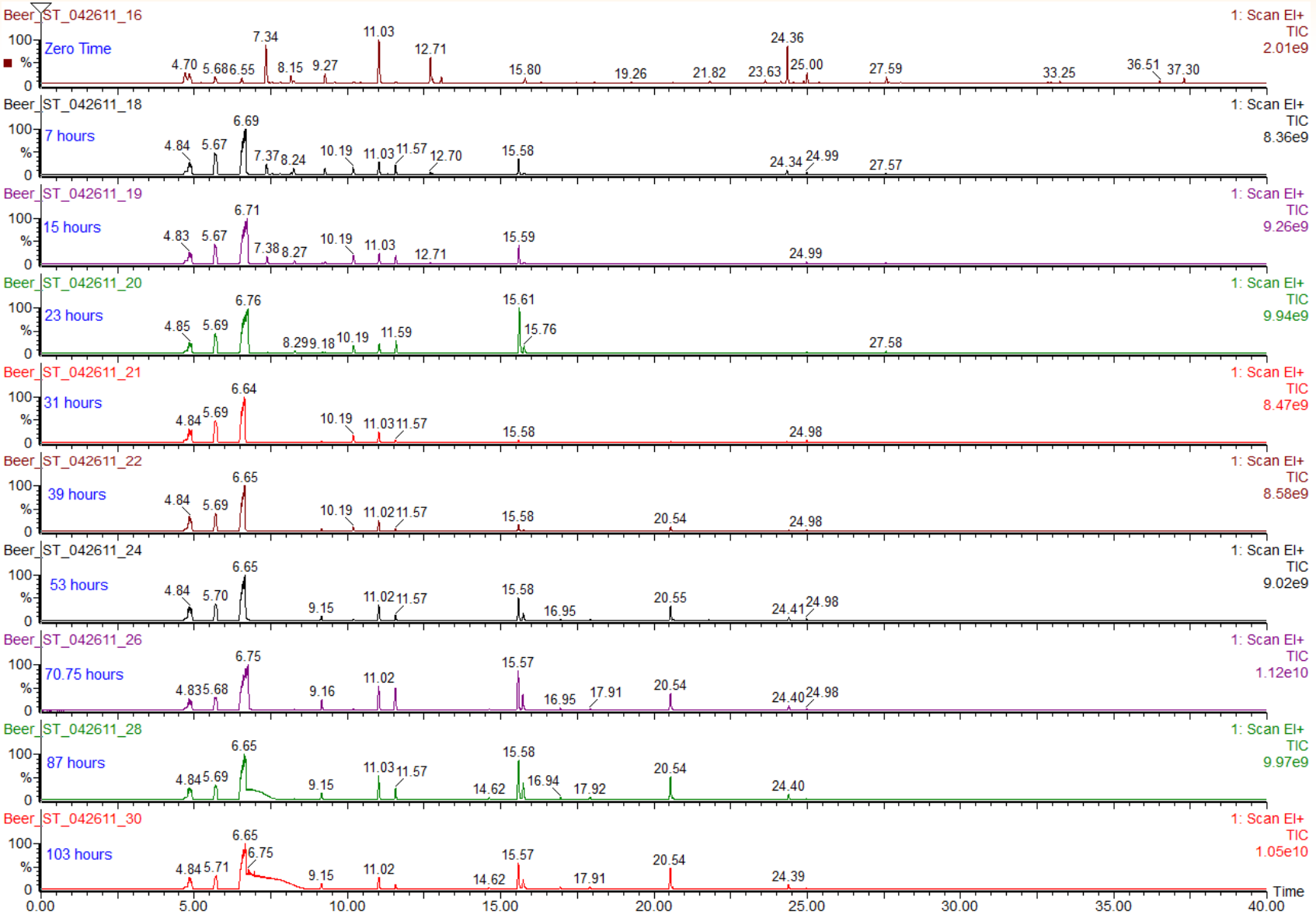


Data Courtesy of Long Trail Brewery, VT

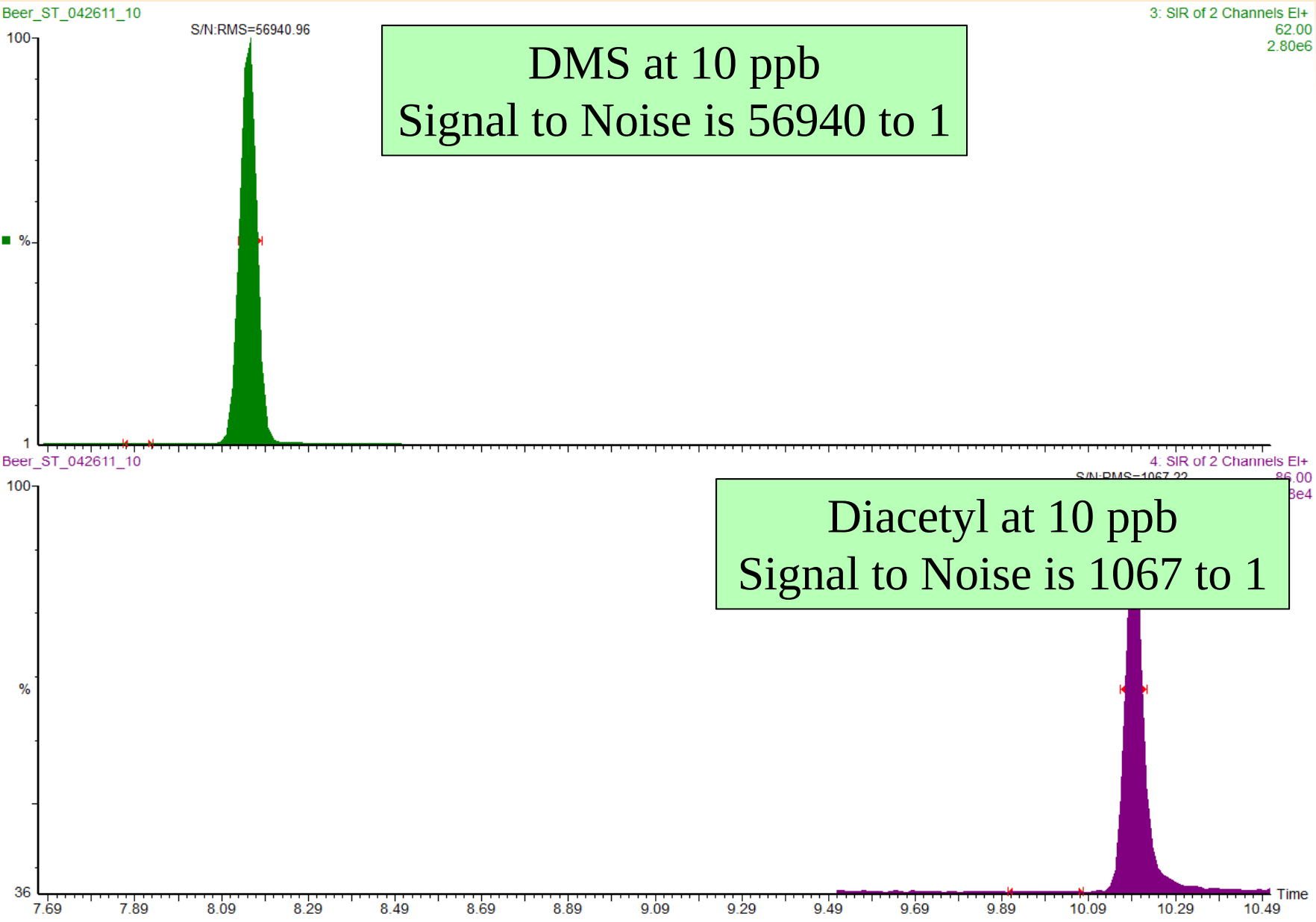
The ‘Profile’ Beer: American Pale Ale

- Grains
  - Maris Otter Pale Malt
  - Munich Malt
  - Crystal Malt
- Hops
  - Chinook
  - Centennial
  - Amarillo
  - Nelson Sauvin
- Yeast
  - SafAle American Ale 05 dry yeast, no starter
- O.G.
  - 1.058
- IBU
  - 45
- Process
  - Infusion mash at 67°C
  - Fermentation at 19-20°C

Beer Compostion Changing with Time

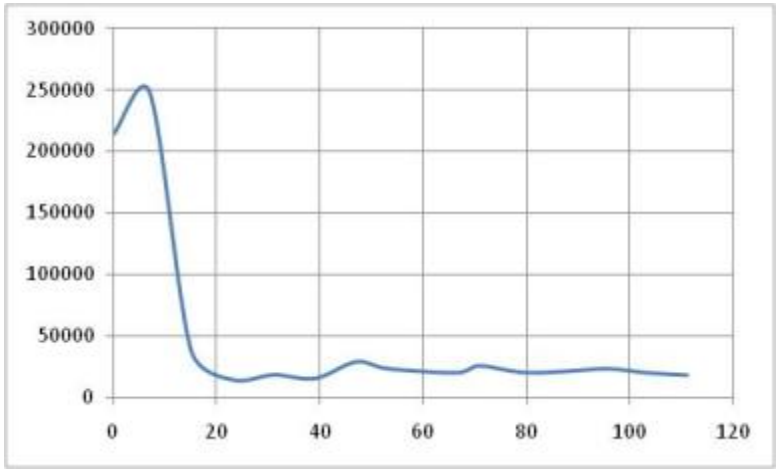


Sensitivity is extremely high by HS Trap/GC/MS

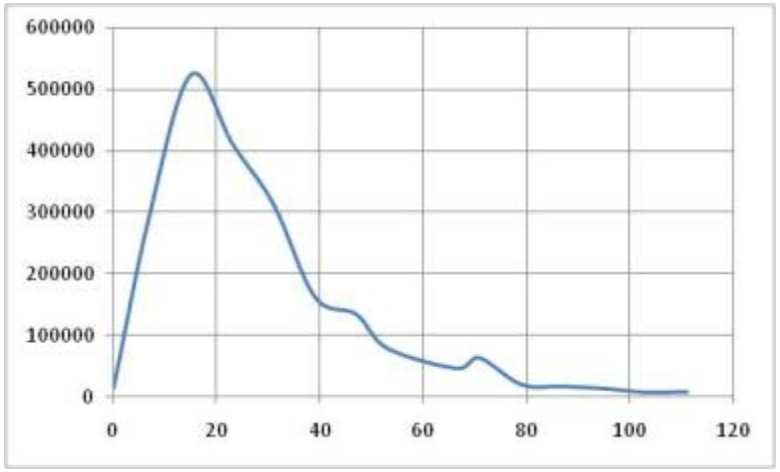


Activity of Two Components over 111 Hours of Sampling

Dimethyl Sulfide (DMS)



2,3-Butanedione (Diacetyl)



Plot: Detector Response versus Time

Time Interval: Every Eight Hours

Typical Hop Composition

Component	%
Vegetative Material (cellulose, lignin, etc.)	40
Proteins	15
Soft Resins	5-23
Hard Resins	1-2
Water	10
Ash	8
Lipids, Wax, Pectin	5
Tannins	4
Monosaccharides	2
Essential Oils	0.5 to 2

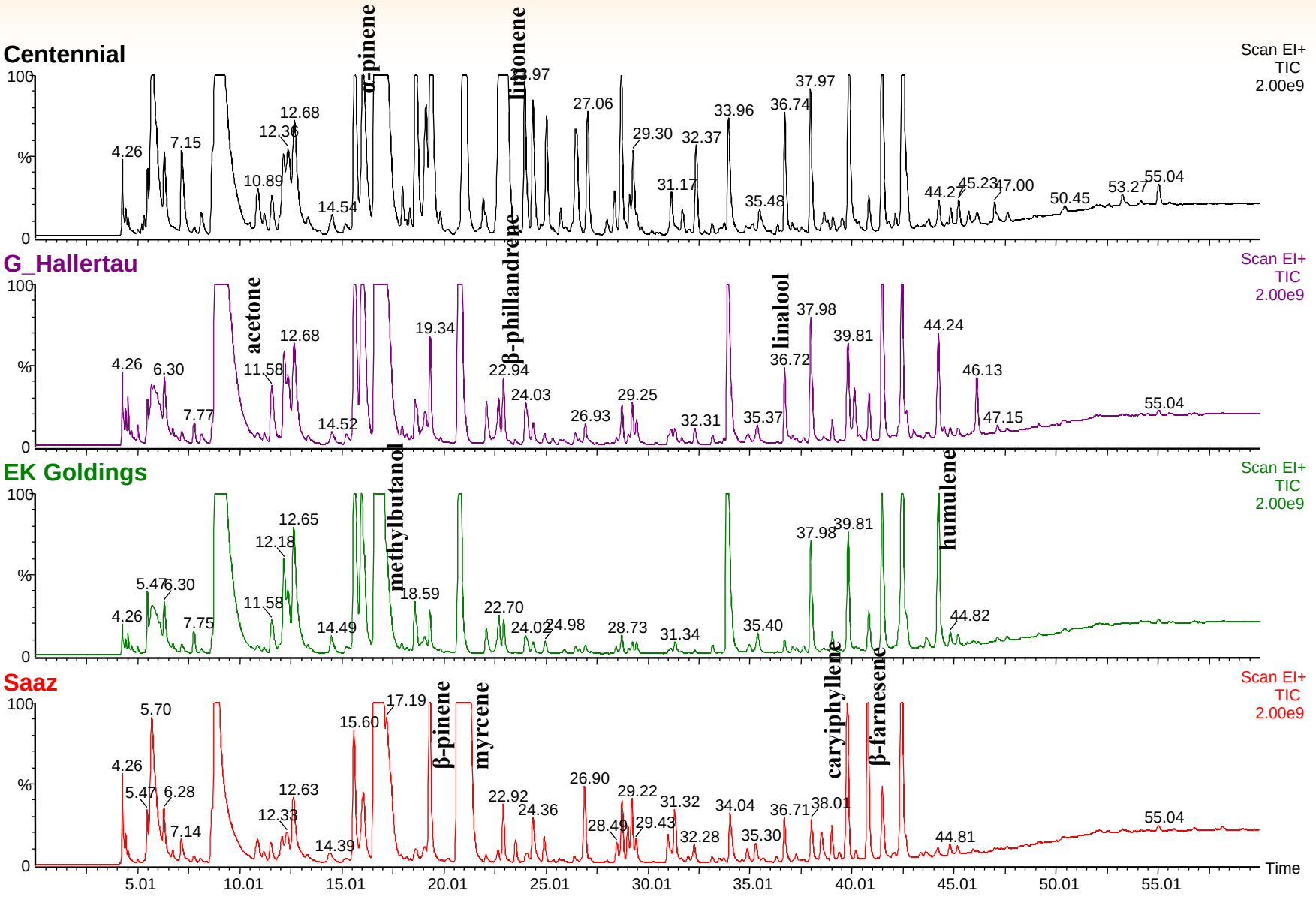
Bittering Compounds

Flavor/Aroma Compounds

Why Analyze Hops

- QC – are they any good or have they aged or oxidized?
- Development – how do hops differ from each other and can we predict the effects of substitution?
- Correlating with final product – what happens to the hops during brewing?

Some key peaks identified

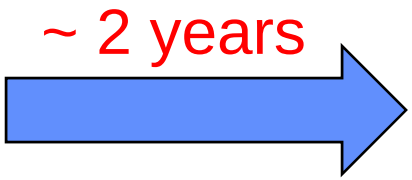


Beer Souring

Brettanomyces

Lactobacillus

Pediococcus



Lactic Acid

Acetic Acid

Ethyl Acetate

Ethyl Lactate

Other C_2 to C_{12} acids and esters

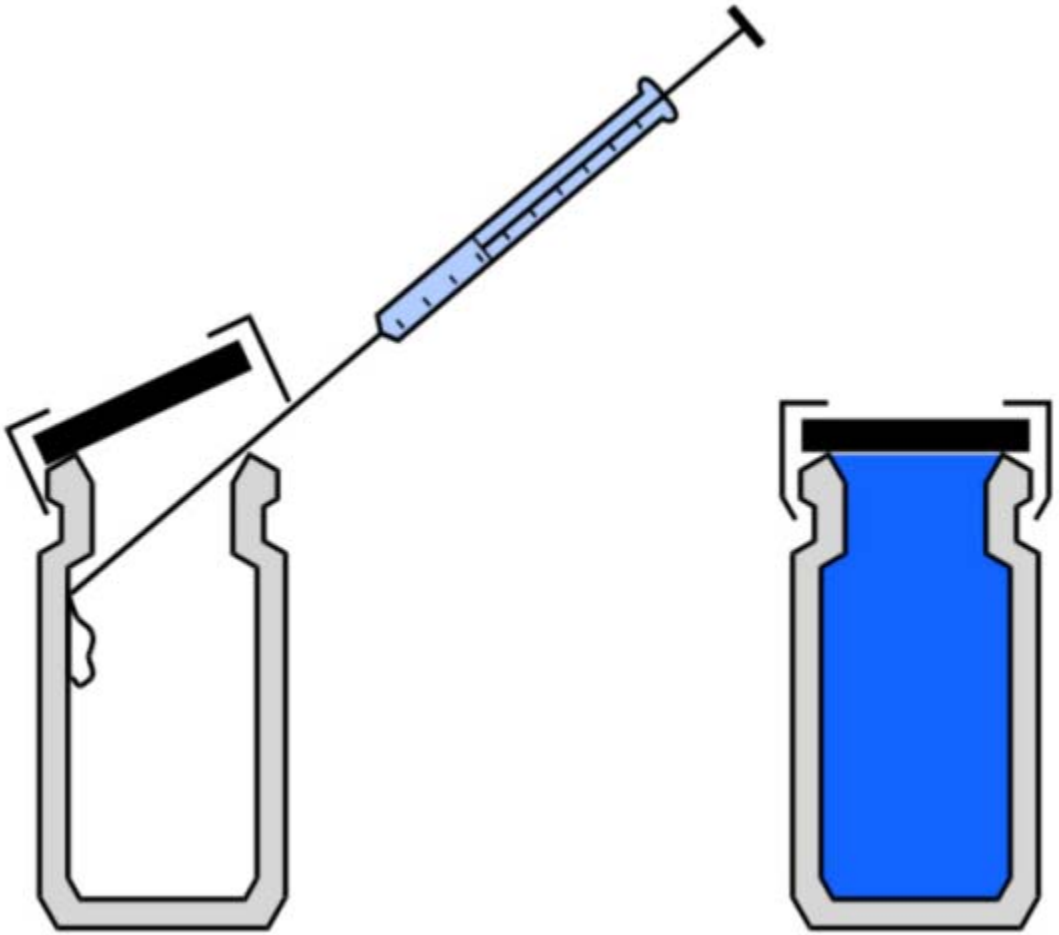
4-Ethylphenol

4- Ethylguaiacol

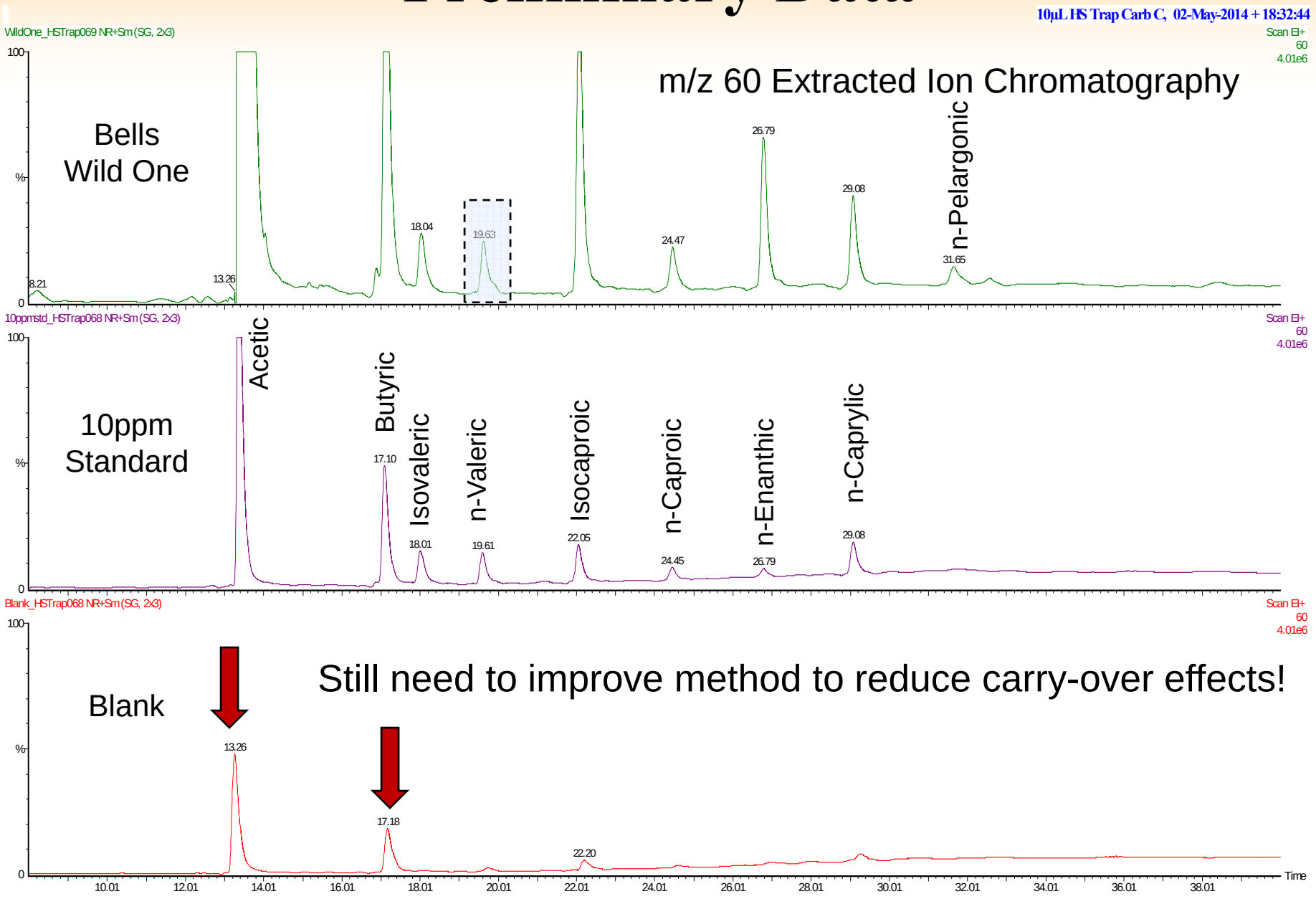
Partition Coefficients for Aliphatic Acids

Acid	K_W	% in HS with 1mL Sample
Acetic (C2)	75,858	0.0013
Propionic (C3)	53,703	0.0019
Butyric (C4)	40,738	0.0025
Valeric (C5)	28,184	0.0035
Caproic (C6)	20,417	0.0049
Enanthic (C5)	15,849	0.0063
Caprylic (C6)	14,125	0.0071
Pelargonic (C9)	10,471	0.0095
Capric (C10)	7,413	0.0135

Total Vaporization Technique



Preliminary Data



Conclusions

Conclusions

- Provides a tool to characterize beer and hop aroma.
- Provides ability to correlate analytical data against organoleptic perception.
- Headspace sample preparation is very easy.
- In-line trap enhances sensitivity.
- Mass spectrometry is highly sensitive and enables volatile aroma components to be easily identified and quantified.
- Olfactory port provides organoleptic characterization to complement analytical data.
- Sour beer analysis shows great promise. Work will continue.

Acknowledgements

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Final thought

What’s in your beer?