



**WORLD BREWING CONGRESS**

August 13–17, 2016 • Denver, Colorado, U.S.A.

#ElevateBeer



# Instrumental Evaluation of Hops

Raw hop analyses

+

Hops in beer analyses





# The range of analyses

## Raw Hops

- How to analyze bitterness potential and total oil

## Beer

- How to analyze hop bitterness in wort/beer
- How to analyze hop aroma in beer/ wort





# Chemical composition of hops

<b>Principle Components</b>	<b>Concentration (%w/w)</b>
Cellulose-lignins	40.0 - 50.0
Protein	15.0
Alpha acids	2.0 - 17.0
Beta acids	2.0 - 10.0
Water	8.0 - 12.0
Minerals	8.0
Polyphenols and tannins	3.0 - 6.0
Lipids and fatty acids	1.0 - 5.0
Hop essential oil	0.5 - 3.0
Monosaccharides	2.0
Pectins	2.0
Amino acids	0.1



European Brewery Convention *Hops and Hop Products, Manual of Good Practice*;  
Getranke - Fachverlag Hans Carl: Nurnberg, Germany, 1997.





# Constituents leading to potential bitterness

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# ASBC Methods of Analyses

Method	Method Description	Instrument	Instrument Price (approx.)	Consumables Price per run (approx.)	Time per run (approx.)
Hops-6	Hops 6. $\alpha$ -and $\beta$ -Acids in Hops and Hop Pellets	spectrophotometer	~ \$6,000-10,000	~\$5	~30 min
Hops-14	High performance liquid chromatography	HPLC	~ \$10,000-40,000	~\$6	~15 min

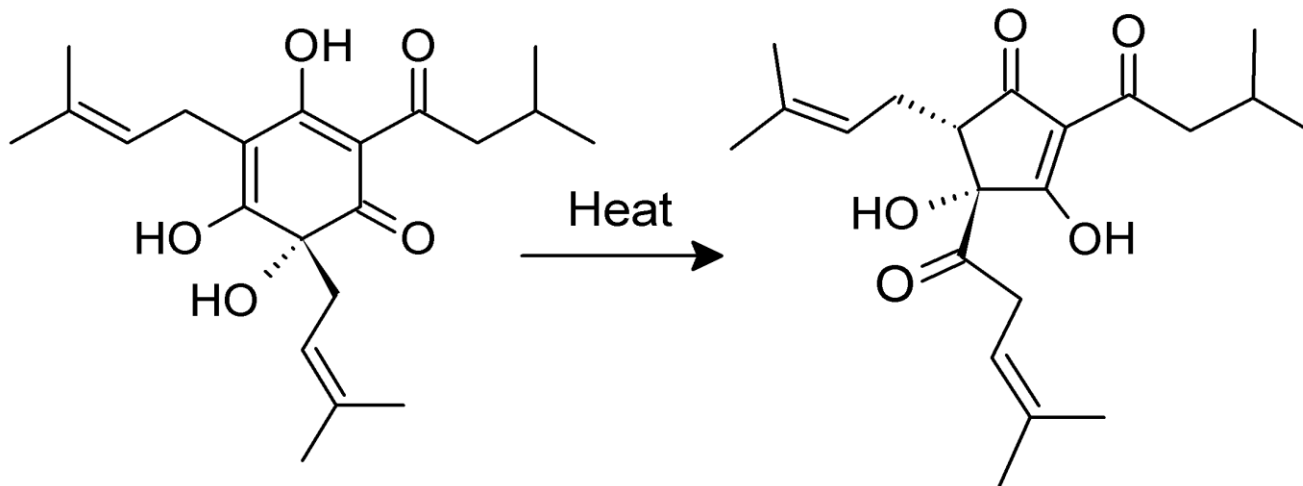
## Hopsteiner Cascade Bitterness Specifications

Bitter Components	
Alpha-Acid %	4.5 - 7
Beta-Acid %	4.5 - 7
Co-Humulone % rel.	33 - 40
Hard Resins : Alpha-Acid	0.27 - 0.56

**Resource:** <http://methods.asbcnet.org/toc.aspx>



# Iso-alpha acids are the most important contributors to bitterness in beer



Alpha acid: Humulone

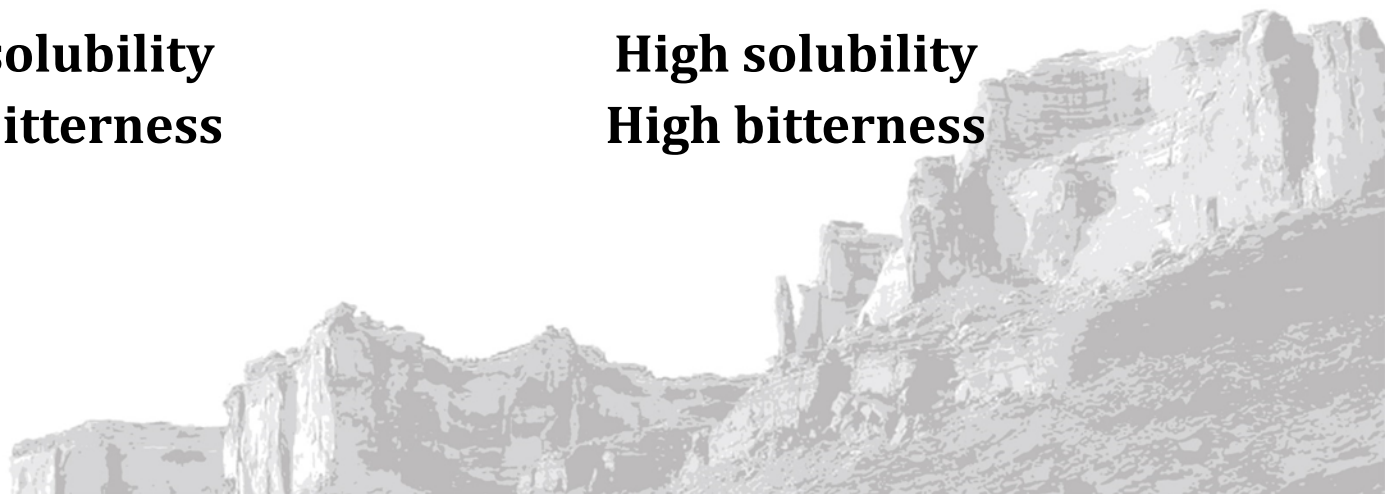
**Low solubility**

**Low bitterness**

Iso-alpha acid: isohumulone

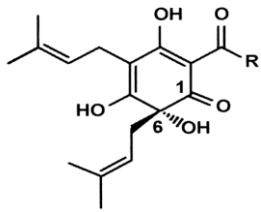
**High solubility**

**High bitterness**

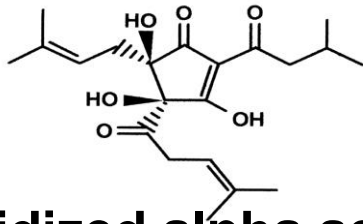




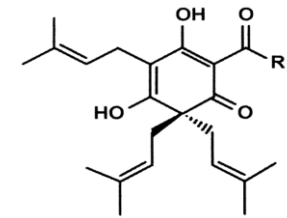
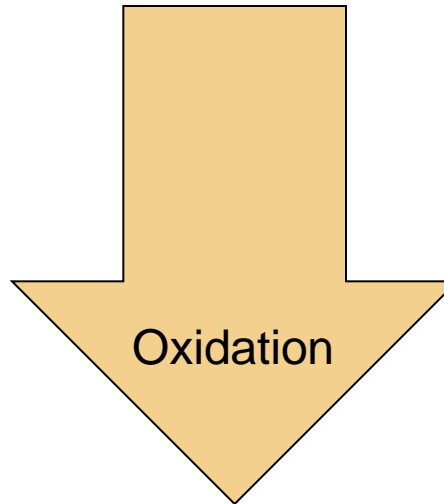
# Other sources of bitterness hop acid oxidation



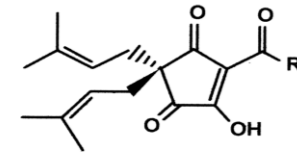
**$\alpha$  acid - Humulone**



**Oxidized alpha acid  
Humulinone**



**$\beta$  acid - Lupulone**



**Oxidized beta acid  
Hulupulone**



# Lead conductance value for measuring alpha acids in hops

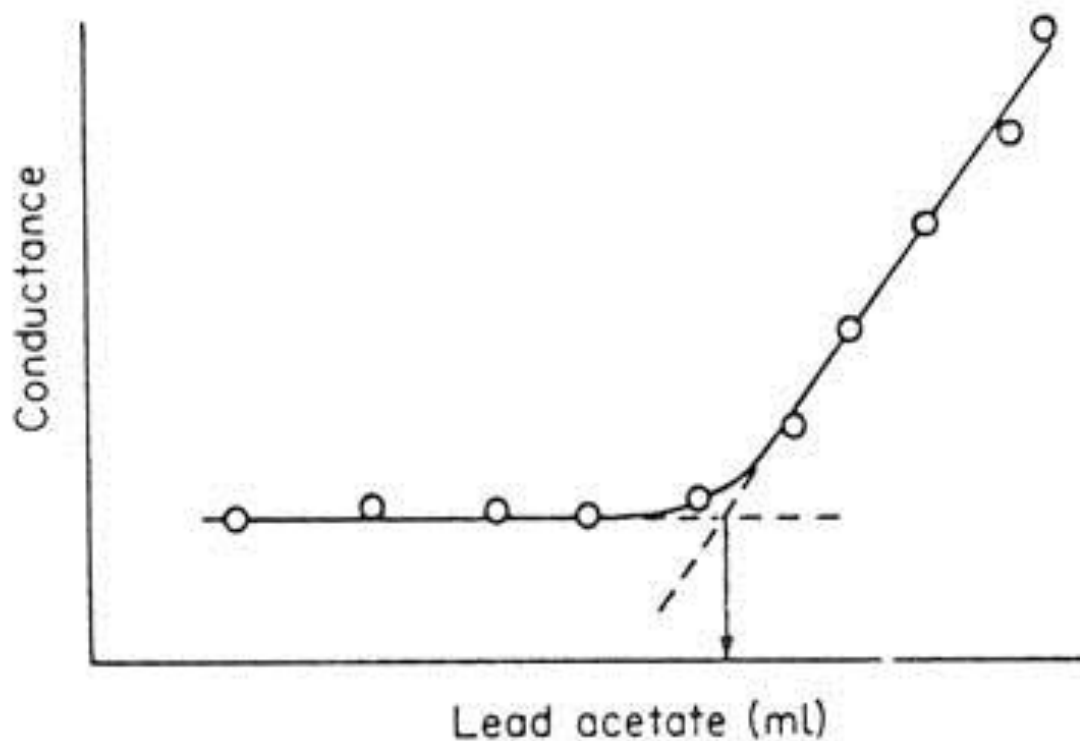


Fig. 2.4 Conductometric titration of  $\alpha$ -acids.

Stevens, 1987

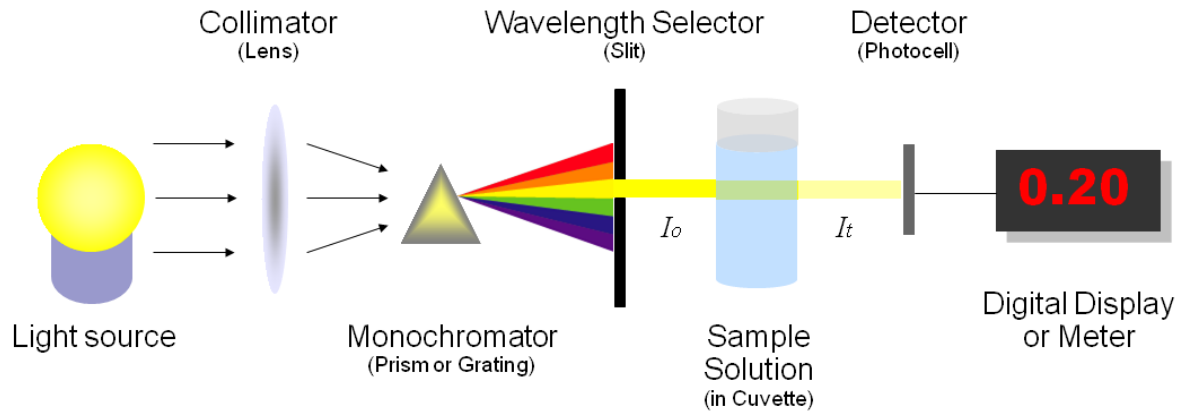


# Spectrophotometry

What is a spectrophotometer?



What is happening under the hood?



# UV spectra for methanol extracts of hops

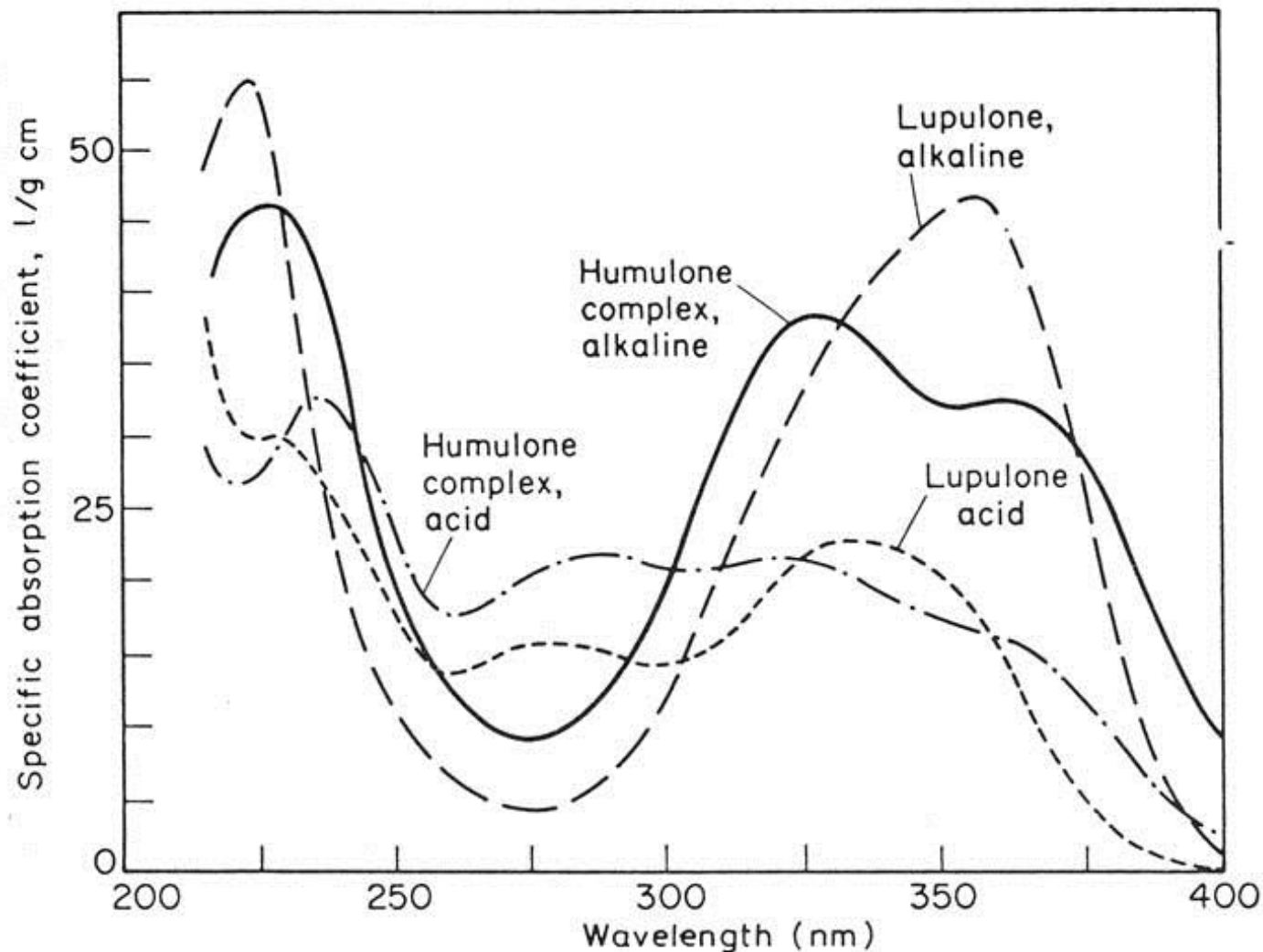
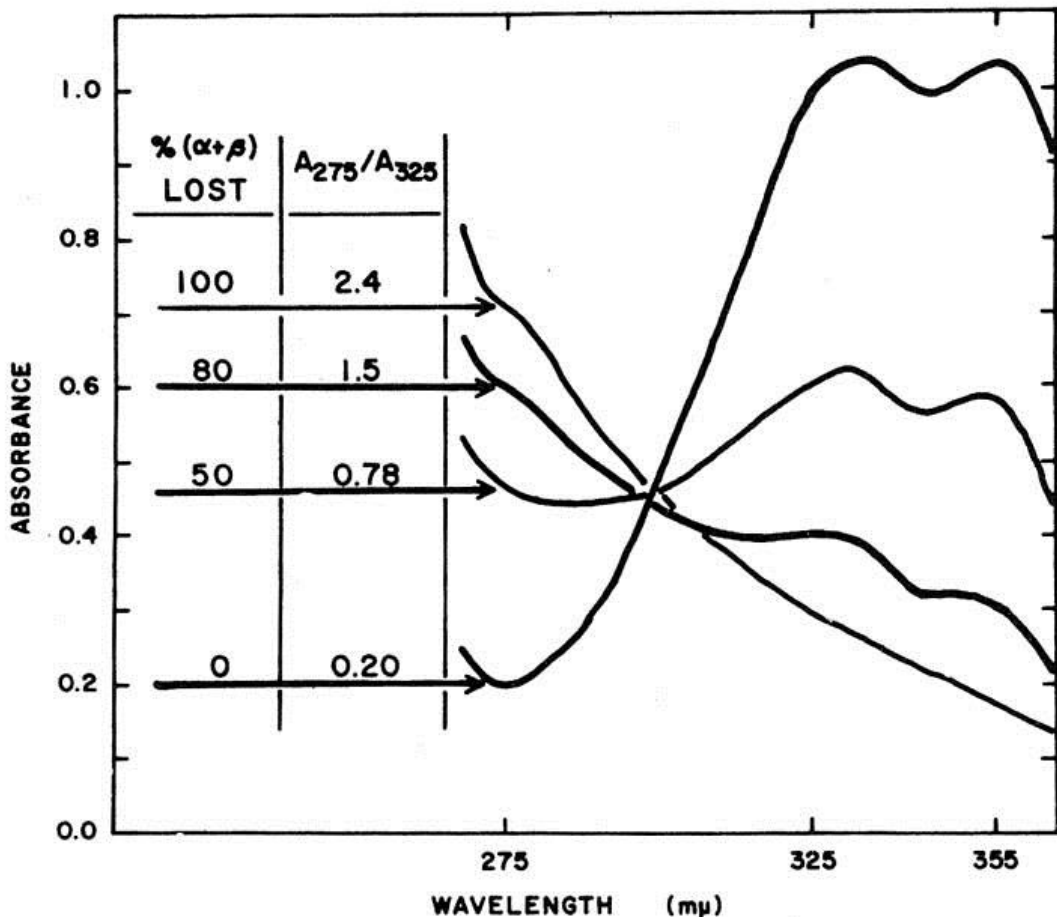


Fig. 2.5. Absorption spectra of lupulone and humulone complex in acidic (0.002 N) and alkaline (0.002 N) methanol.

Stevens, 1987

# The Hop Storage Index (HSI)



$$(HSI) = A_{275}/A_{325}$$

Figure 1. Typical spectra of alkaline methanol solutions obtained from hops in various stages of deterioration.  $A_{325}$  decreases as hop acids are oxidized and  $A_{275}$  increases as oxidation products accumulate, resulting in proportional increases in  $A_{275}/A_{325}$ .



# Maturity - HSI & oils

Maturity Series of the Hop Variety Bullion<sup>a</sup>

Date	ml oil/ 100 g hops	α-acids (%)	β-acids (%)	A <sub>275</sub> /A <sub>325</sub>		
				Found	Theor- etical	Excess
3 Aug. '66	0.18	7.9	4.7	0.22	0.16	0.06
6 Aug. '66	0.22	8.5	4.7	0.23	0.19	0.04
9 Aug. '66	0.42	10.1	5.4	0.22	0.20	0.02
12 Aug. '66	0.62	9.4	5.0	0.24	0.20	0.04
15 Aug. '66	1.15	11.3	6.0	0.22	0.21	0.01
18 Aug. '66	1.54	11.7	5.6	0.22	0.20	0.02
21 Aug. '66	1.71	10.8	4.3	0.22	0.20	0.02
25 Aug. '66	2.44	11.7	5.0	0.24	0.20	0.04
28 Aug. '66	2.67	10.9	5.6	0.26	0.20	0.06
31 Aug. '66	2.92	10.6	4.5	0.25	0.20	0.05
2 Sept. '66	3.30	11.3	5.6	0.25	0.21	0.04

<sup>a</sup>Oil contents were determined by the method of Wright and Connery (6). α- and β-acids were determined by the spectrophotometric method (A.S.B.C.). Results are adjusted to a common basis of 8% moisture in the hops.

Lickens, et al., 1970

# HSI data 1970

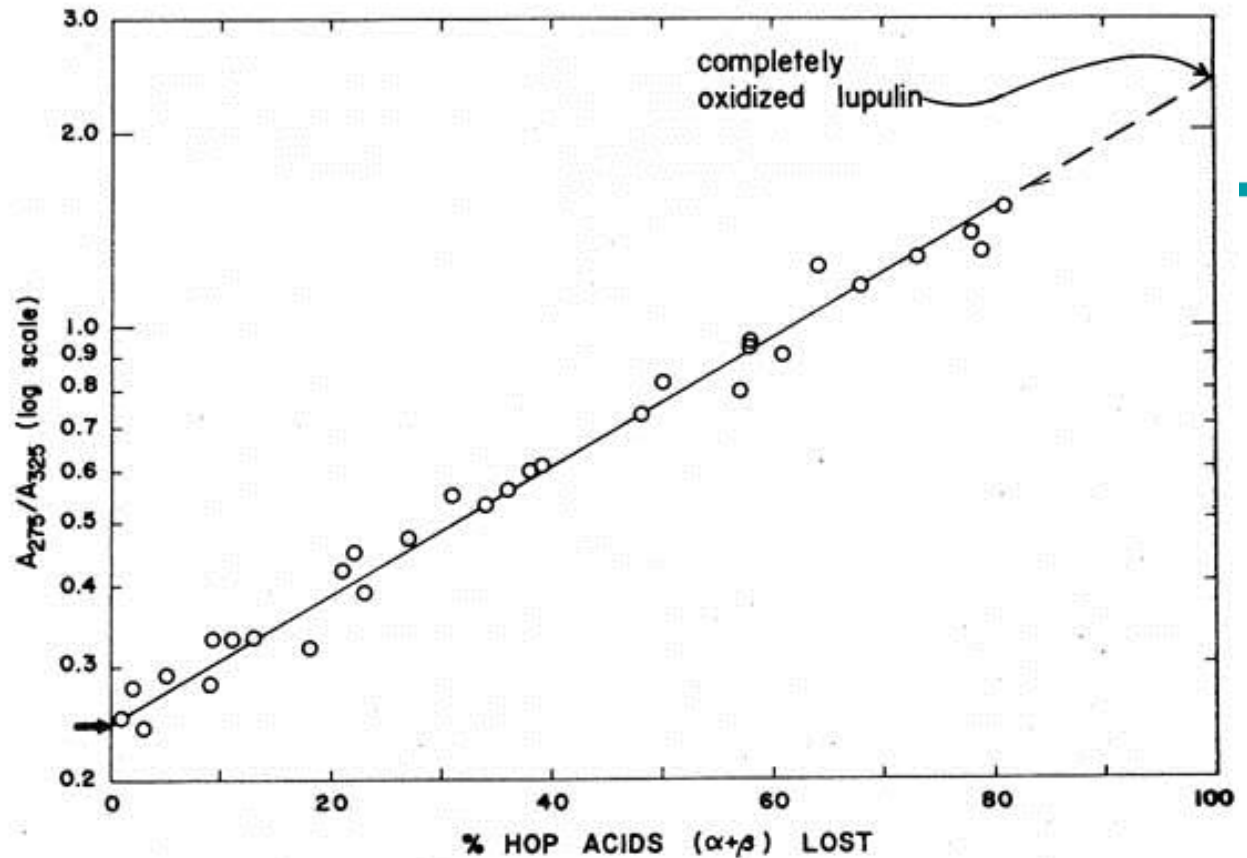
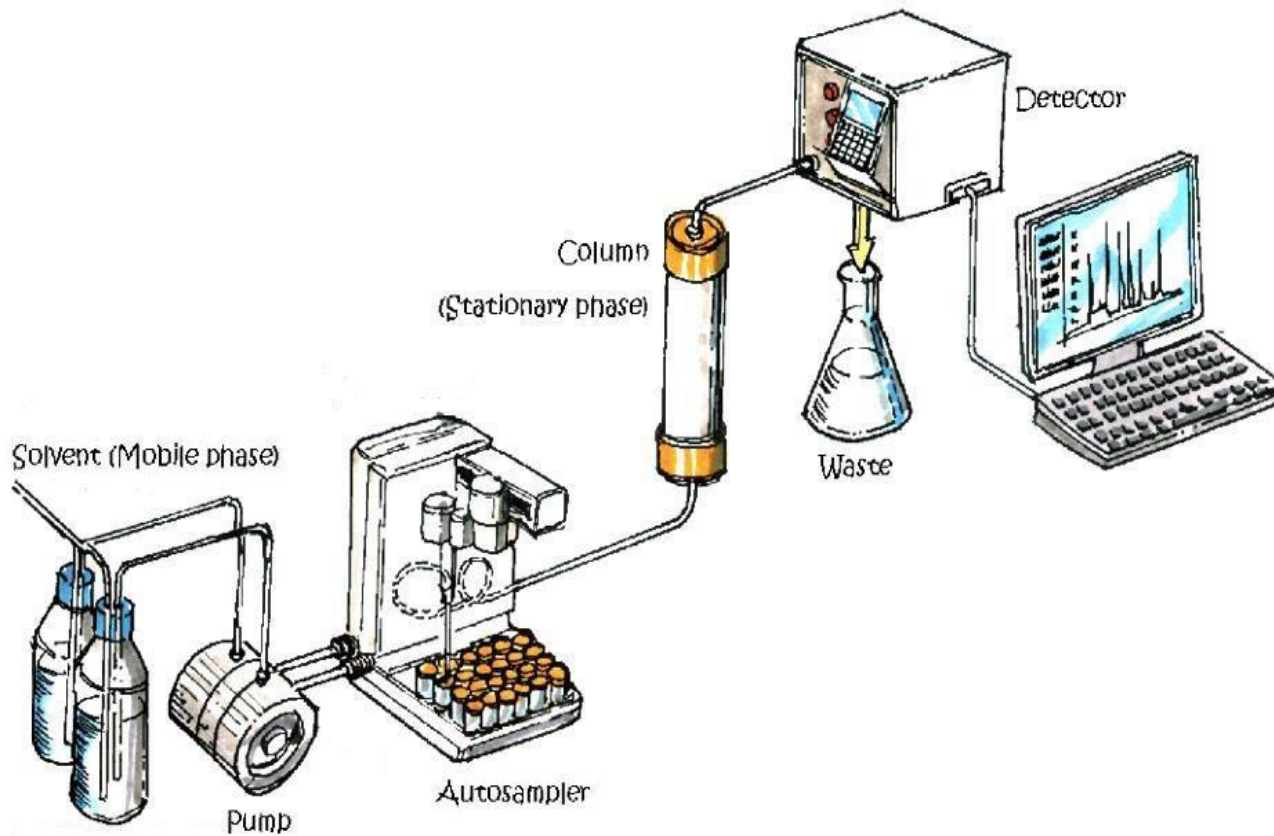


Figure 2. Relationship of deterioration of hop acids to increase of  $A_{275}/A_{325}$ . Nine varieties stored 3, 6, and 10 months are represented. The arrow at  $A_{275}/A_{325} = 0.24$  is the average initial value for all varieties. Lupulin was held at elevated temperatures until no further change took place in its absorption spectrum:  $A_{275}/A_{325} = 2.5$  and represents 100% loss.

Lickens, et al., 1970

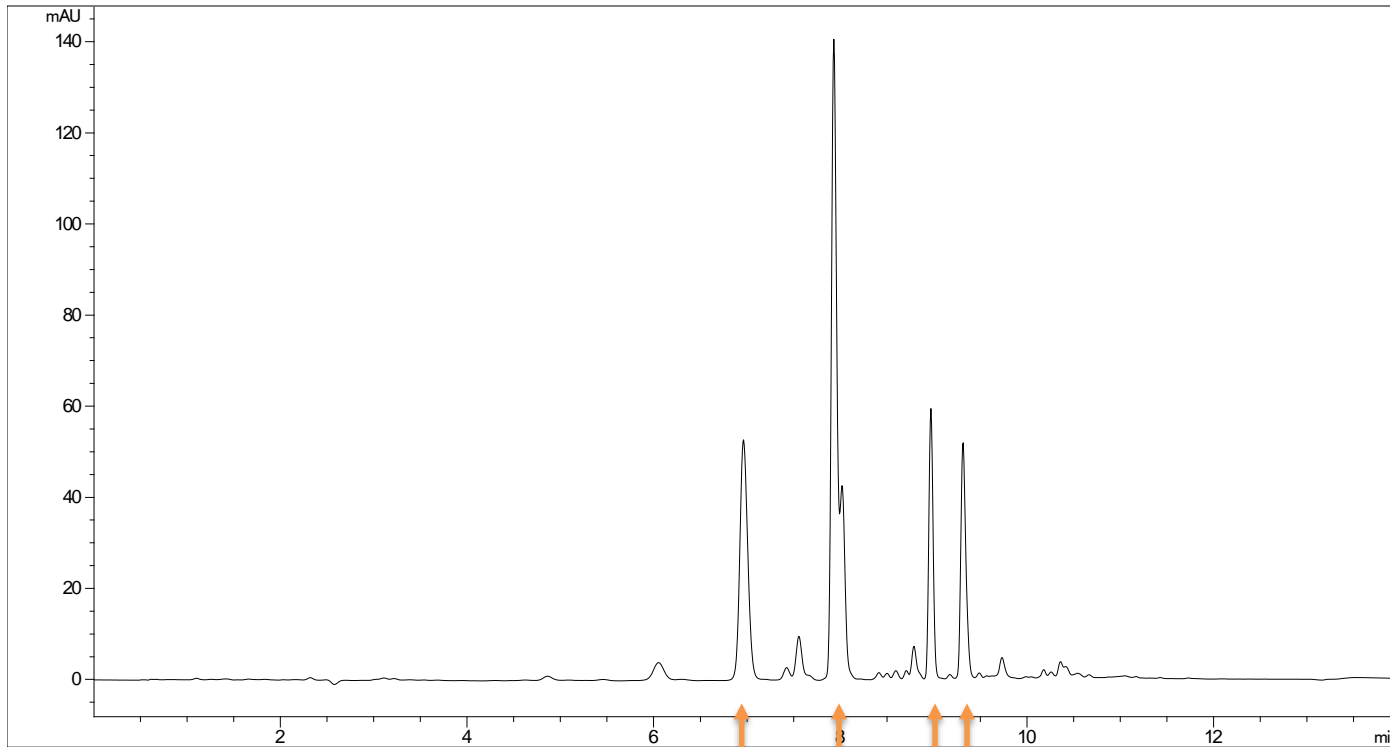


# High Performance Liquid Chromatography (HPLC)



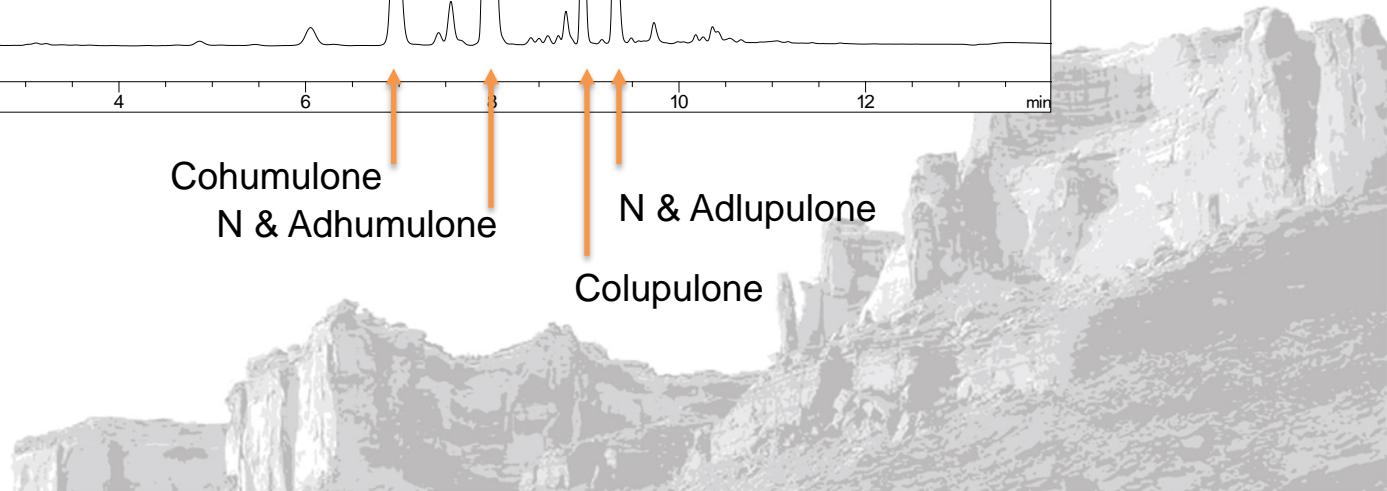


# Hop acid standards



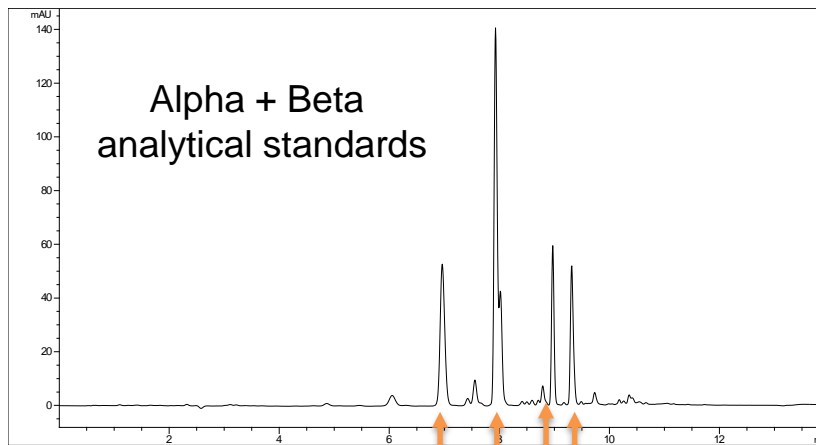
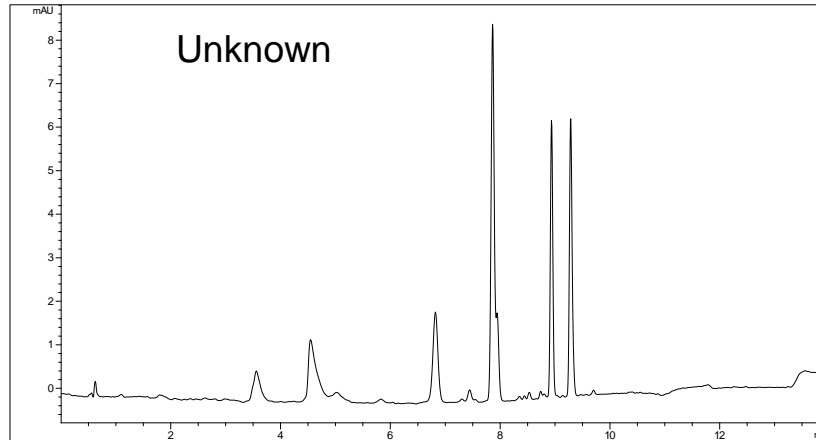
Cohumulone  
N & Adhumulone

N & Adlupulone  
Colupulone





# Compare unknown to standard



Cohumulone  
N & Adhumulone  
Colupulone  
N & Adlupulone





# Constituents leading to potential aroma

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# Measuring aroma potential in hops

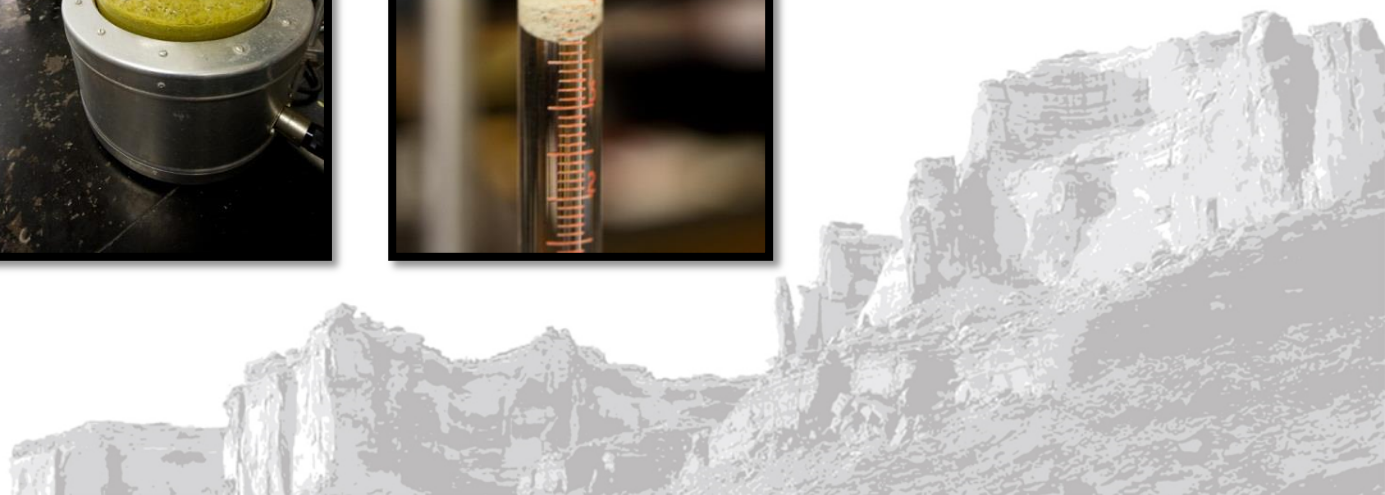
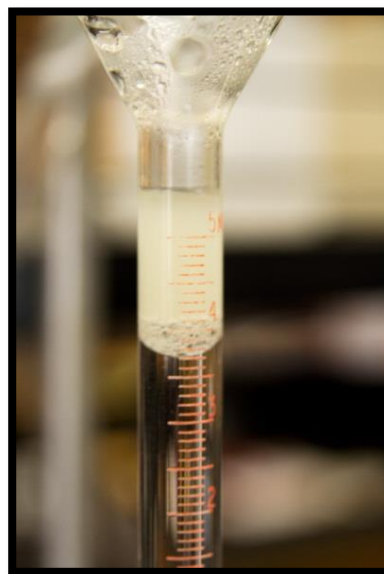
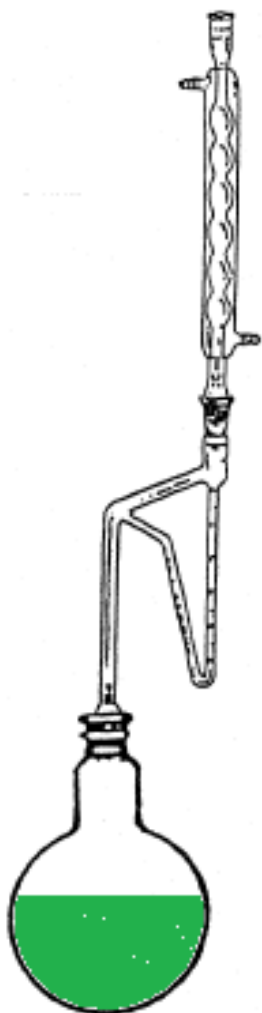
Method	Method Description	Instrument	Instrument Price (approx.)	Price per run (approx.)	Time per run (approx.)
Hops 13	Total Essential Oils in Hops by Steam Distillation	Distillation Equipment	~ \$1,000-3,000	~\$1	~5 hr
Hops 17	Hop Essential Oils by Capillary Gas Chromatography-Flame Ionization Detection	GC-FID	~ \$25,000-50,000	~\$5	~90 min

**Resource:** <http://methods.asbcnet.org/toc.aspx>



# Measuring aroma potential in hops

- Essential oil extracted from hops via hydrodistillation
- Recent research suggest that there are potential issues with thermal conversion of analytes
- Is this really representative of what is in hops?



# Hop oil contribution to beer aroma

- Hydrocarbons
  - Monoterpenes – Myrcene
  - Sesquiterpenes – Caryophyllene, Farnesene
- Oxygenated compounds
  - Terpene alcohols – Linalool, Geraniol
  - Sesquiterpene alcohols – Humulenol
  - Epoxides – Humulene epoxide
  - Other – Caryophyllene oxide
- Sulphur compounds
  - Thioesters
  - Sulfides
  - Other

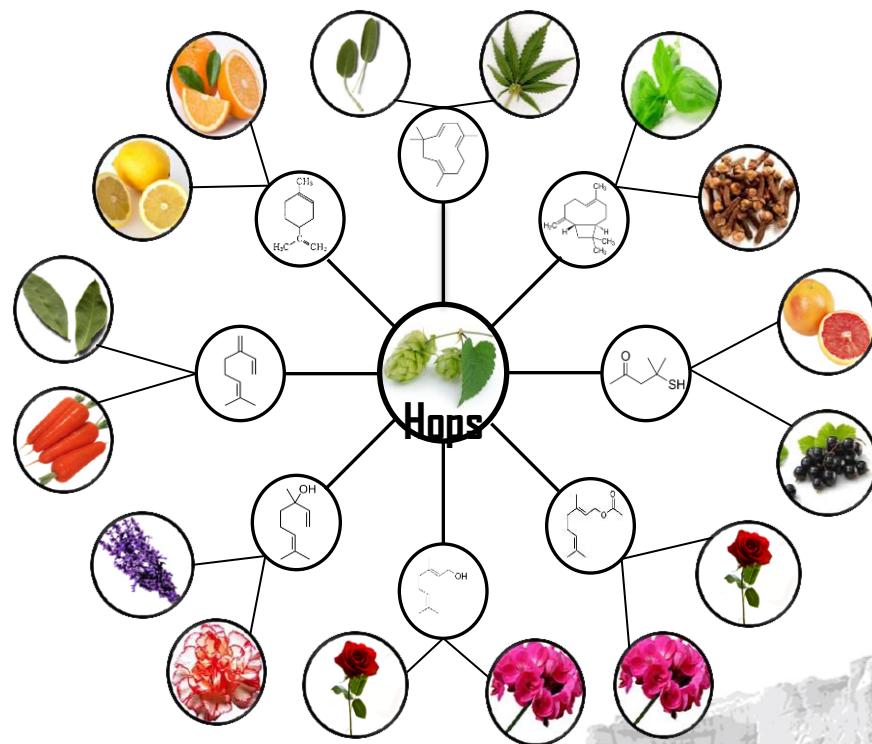
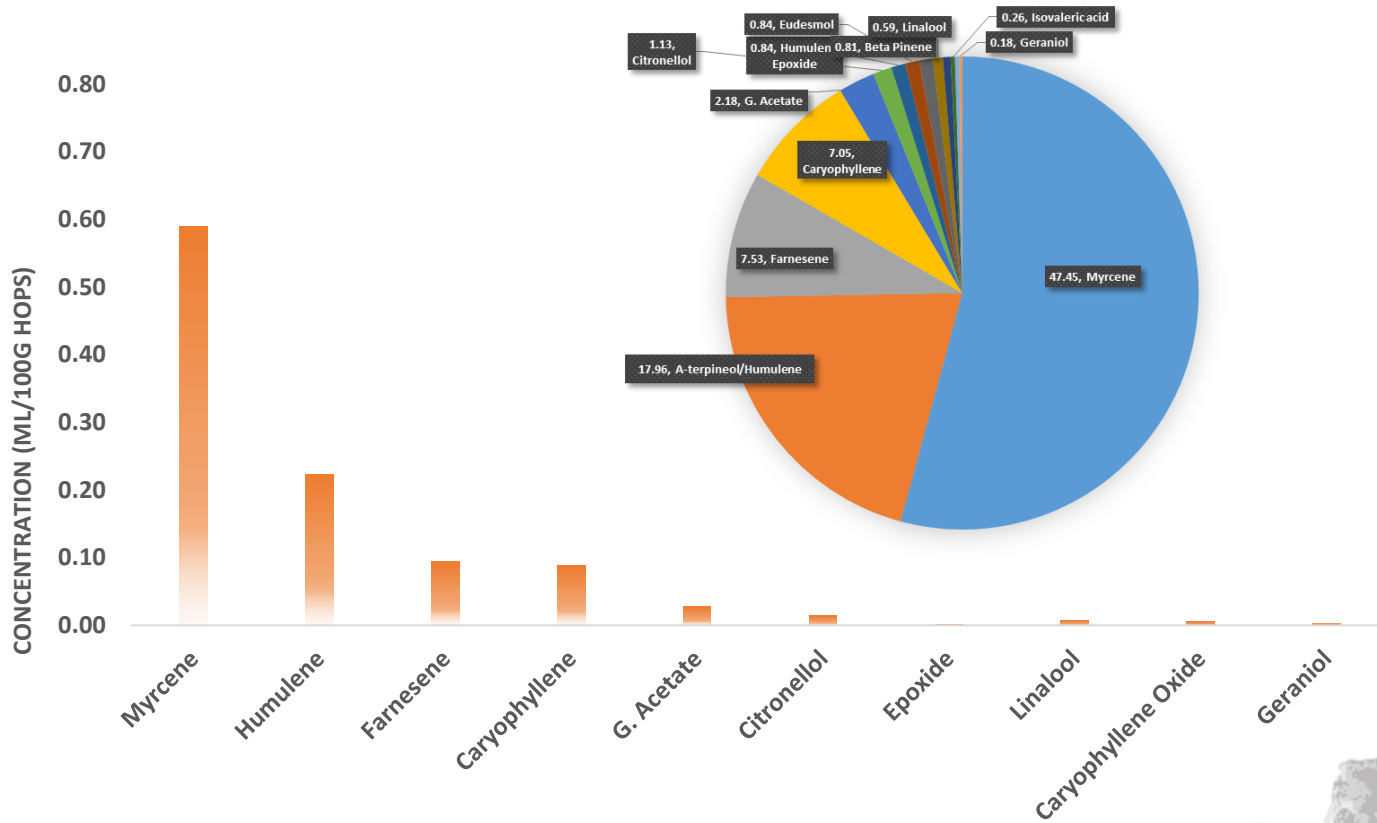


Figure adapted from Victor Algazzali



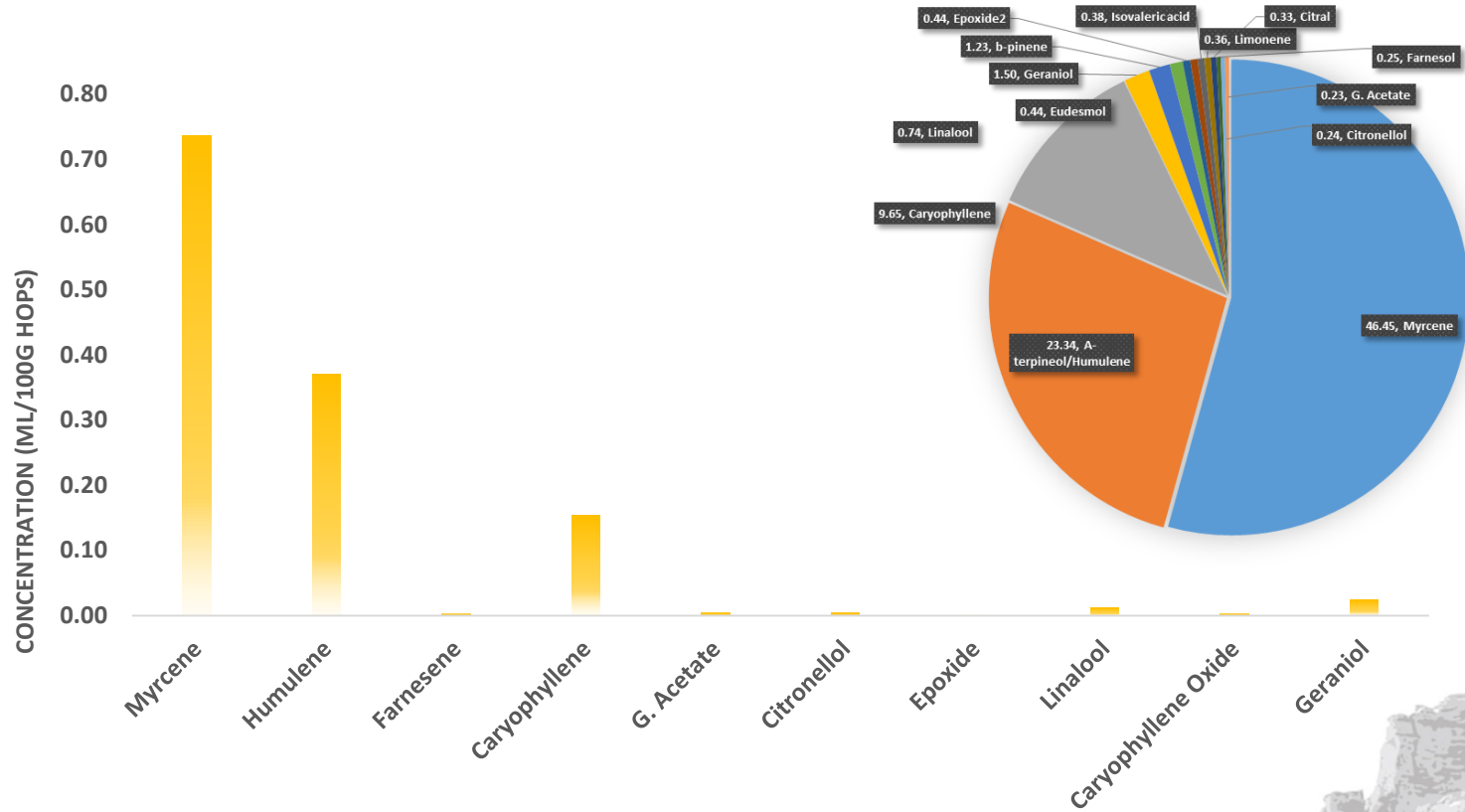
# Cascade Hop Oil Profile (0.7 – 1.4 ml/100g)





# Centennial Hop Oil Profile

(1.5 – 2.3 ml/100g)





# Measuring bitterness in beer

Method	Method Description	Instrument	Instrument Price (approx.)	Consum. price per run (approx.)	Time per run (approx.)
ASBC Wort/ Beer 23 IBU	Beer or Wort Bitterness International bitterness unit	spectrophotometer	~ \$6,000- 10,000	~\$3	~30 min
ASBC Wort/ Beer 23 HPLC	High performance liquid chromatography	HPLC	~ \$25,000- 50,000	~\$2	~15 min

**Resource:** <http://methods.asbcnet.org/toc.aspx>



# Beer 23 – International Bitterness Unit

Liquid-Liquid extraction of bitter compounds from beer

## Beer

### Bitter compounds

Iso alpha acids  
Oxidized hop acids  
Polyphenols

### Non-bitter compounds

Alpha acids

+

## Strong Acid

Adds excess hydrogen ions to solution – protonating all carboxylic acid functional groups

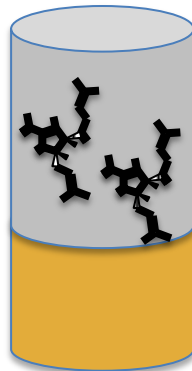
3 N Hydrochloric Acid

+

## Non-Polar Solvent

When functional groups are protonated at a lower pH molecules decrease in polarity

2,2,4 trimethylpentane

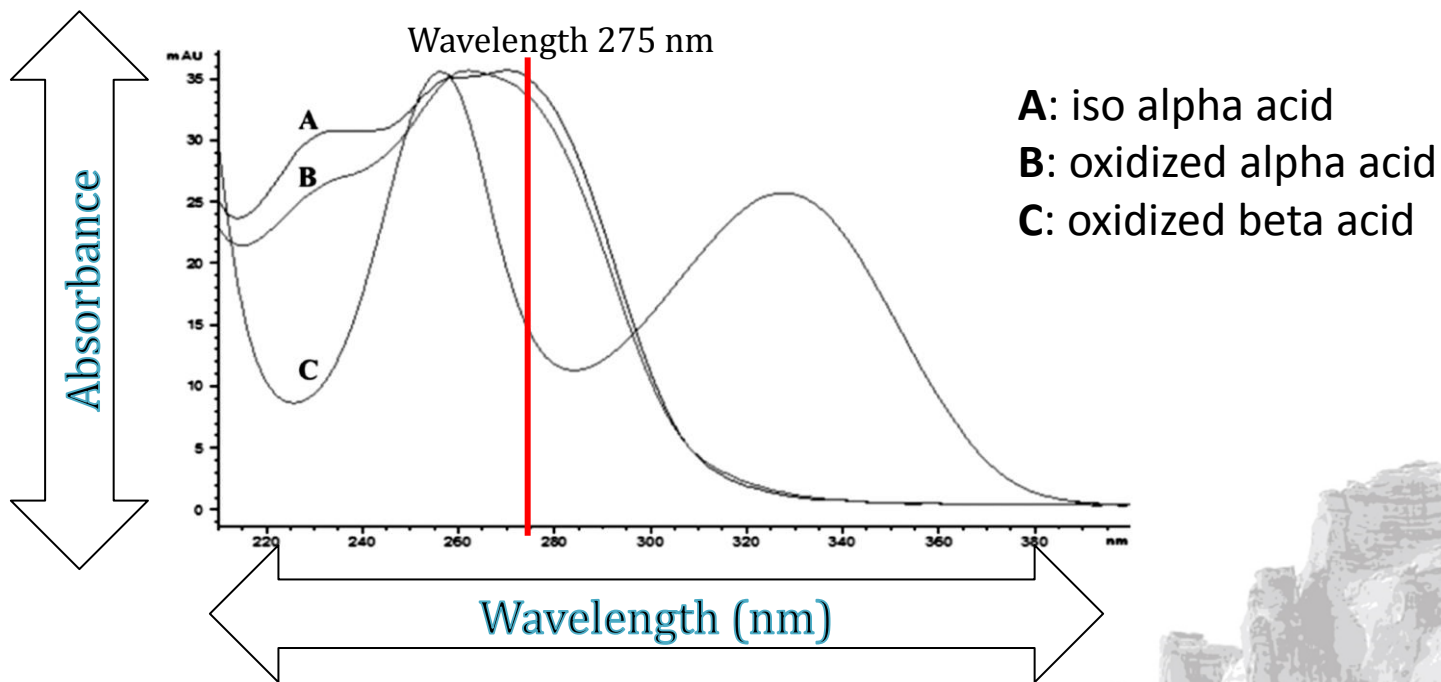






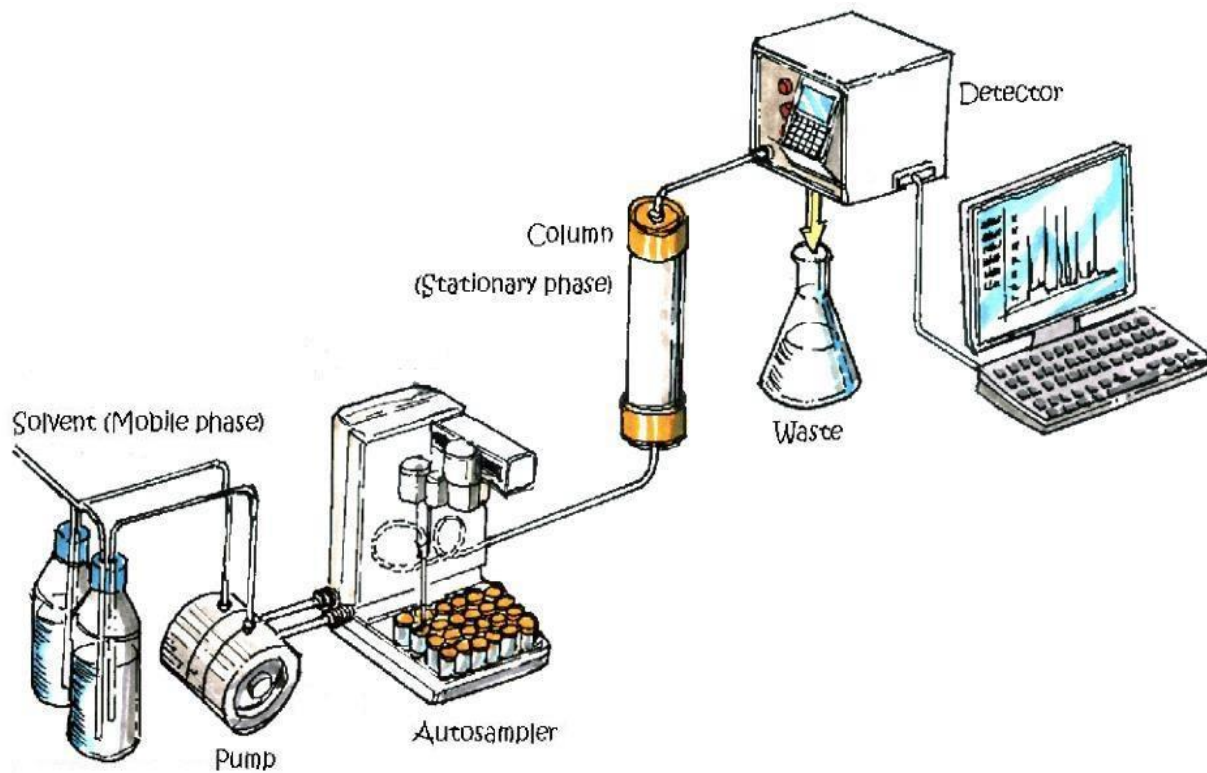
# Beer 23 – International Bitterness Unit

- Measure the absorbance at 275 nm
- Absorbance @275 x 50 = *Bittering Units*  $\neq$  1 ppm iso-alpha acid





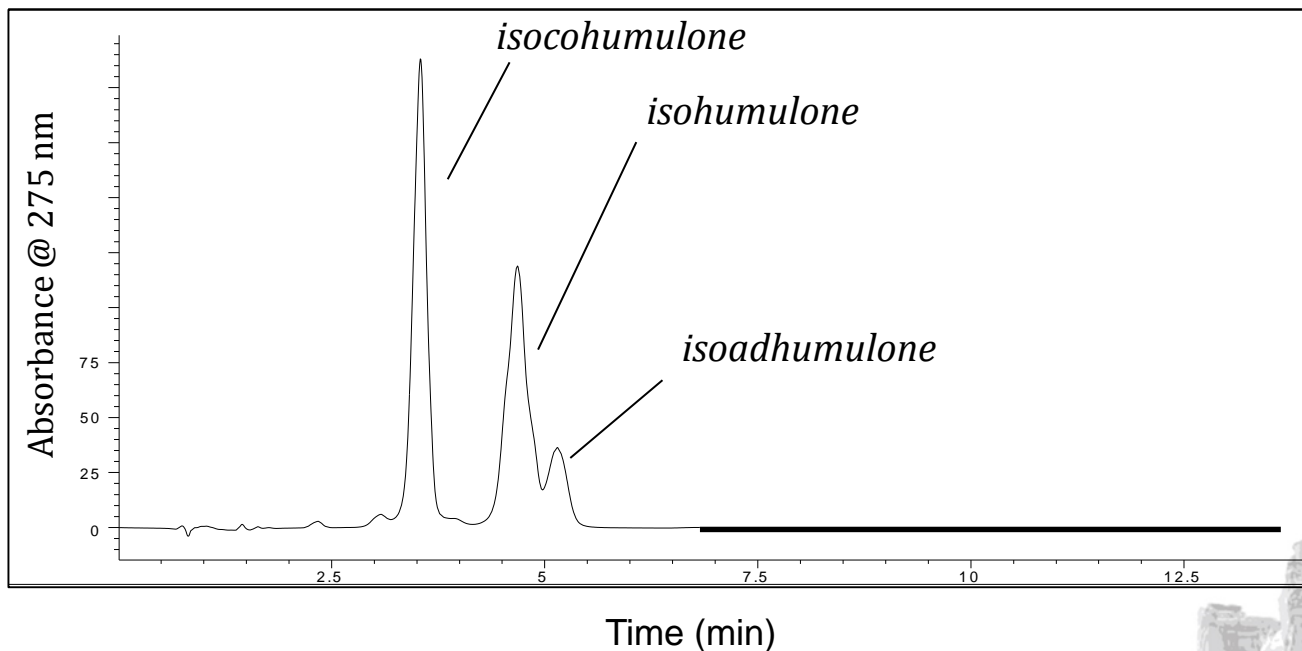
# Beer 23 – Iso-alpha acids via HPLC





# Beer 23 – Iso-alpha acids via HPLC

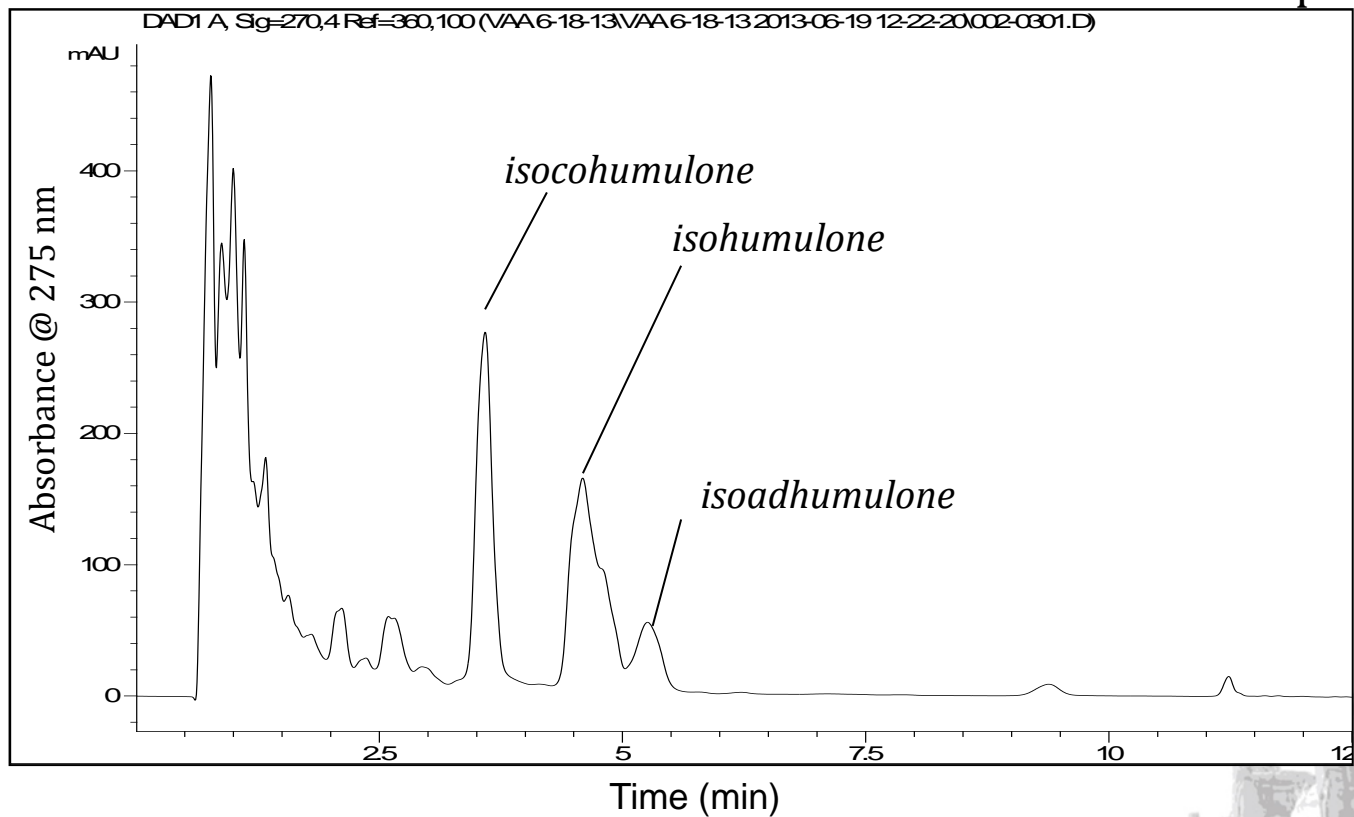
- Measuring Absorbance at 280nm of iso-alpha acids standard produces standards chromatogram
- Standards can be purchased on ASBC website
- Isocohumulone+ isohumulone+ isoadhumulone = total iso-alpha acids (mg/L)





# Beer 23 – Iso-alpha acids via HPLC

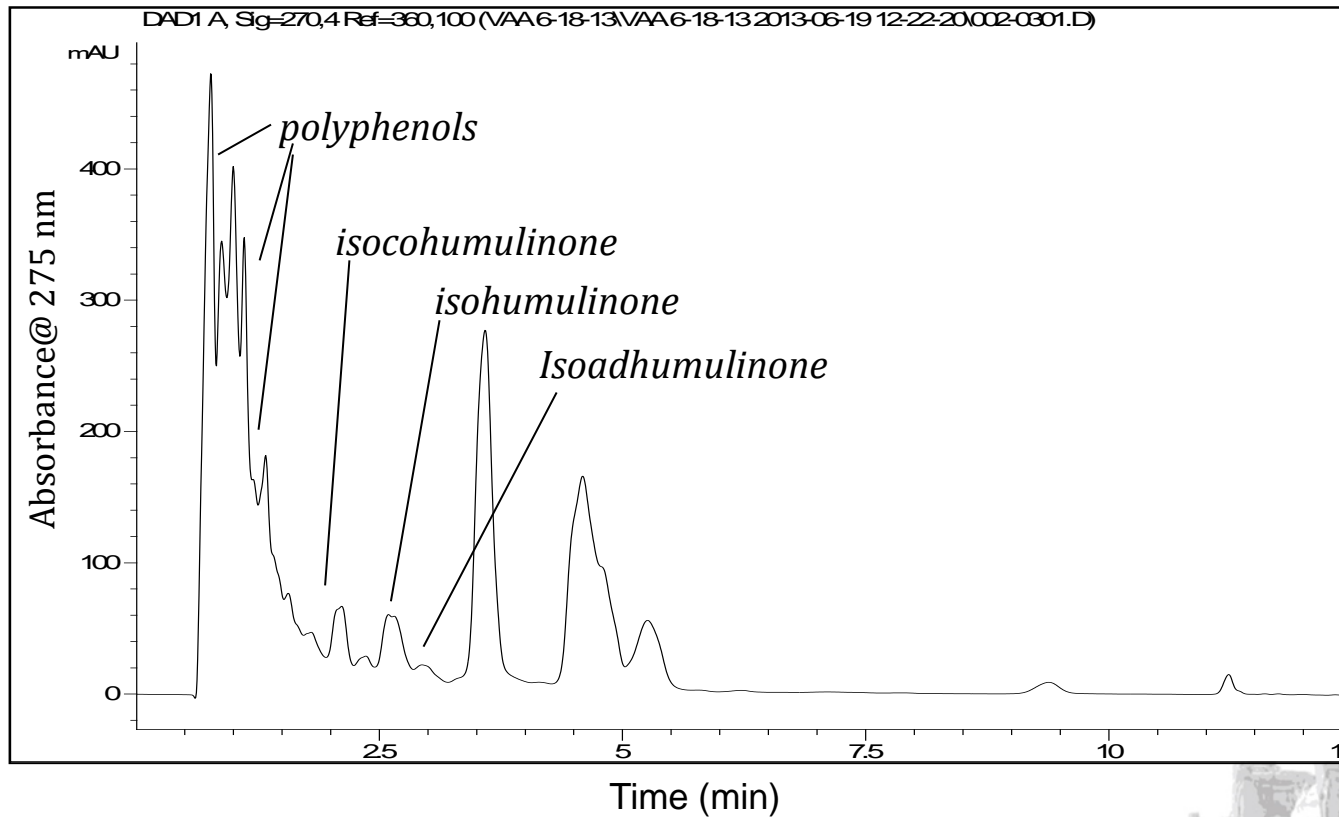
- Compare unknown to iso standard
- Isocohumulone+ isohumulone+ isoadhumulone = total iso-alpha acids (mg/L)





# Beer 23 – Non-iso-alpha acids bitterness

- Can also measure other analytes! You just need appropriate standards!





# Measuring hop aroma in beer

Method	Method Description	Instrument	Instrument Price (approx.)	Price per run (approx.)	Time per run (approx.)
ASBC Beer 48	Headspace Gas Chromatography-Flame Ionization Detection Analysis of Beer Volatiles	GC-FID	~ \$25,000-50,000	~\$10-30	~70 min
ASBC Method in Development	Solid phase Micro Extraction Gas Chromatography-Mass Spectrometry Analysis of Beer Volatiles	SPME GC-MS	~ \$90,000-150,000	~\$30-75	~90 min
Best Case Scenario	Stir Bar Gas Chromatography-Mass Spectrometry Analysis of Beer Volatiles	Stir Bar GC-MS	~ \$125,000-175,000	~\$50-100	~2 hr

**Resource:** <http://methods.asbcnet.org/toc.aspx>

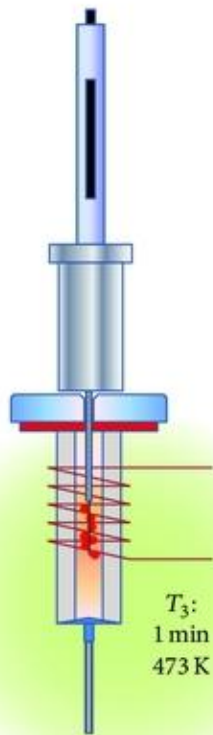
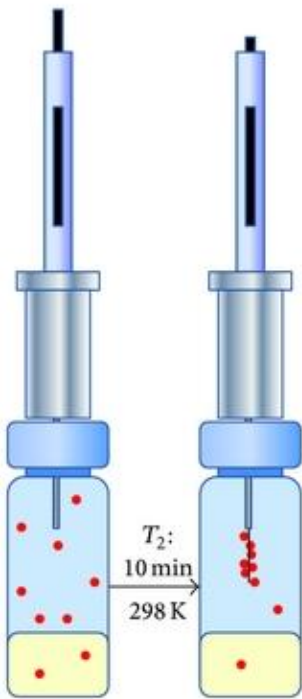


# Hop Aroma Analysis

## Headspace SPME GC-MS

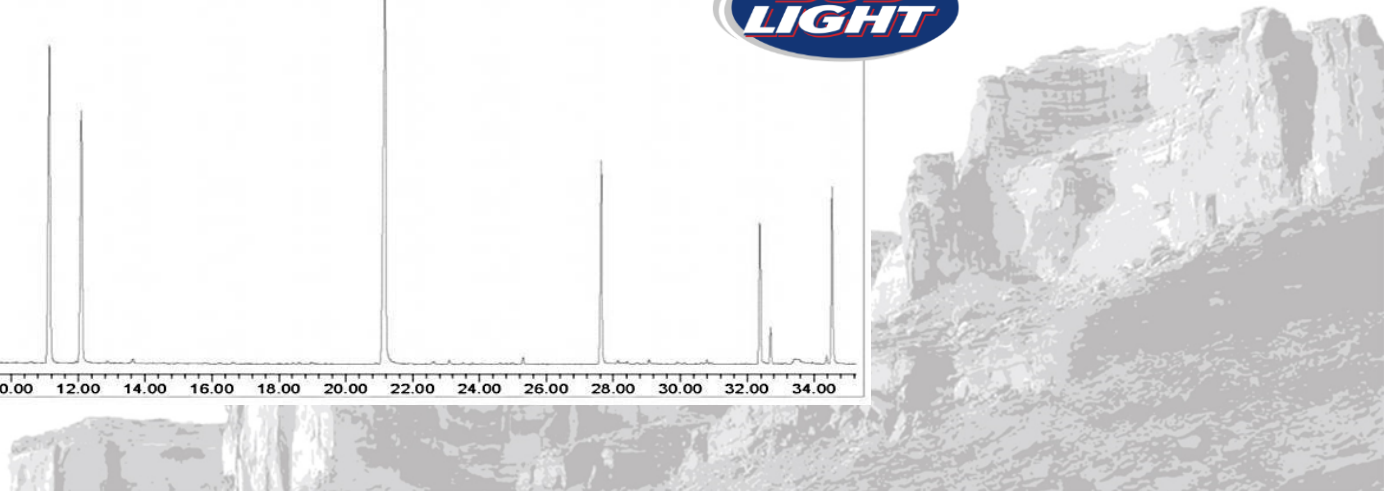
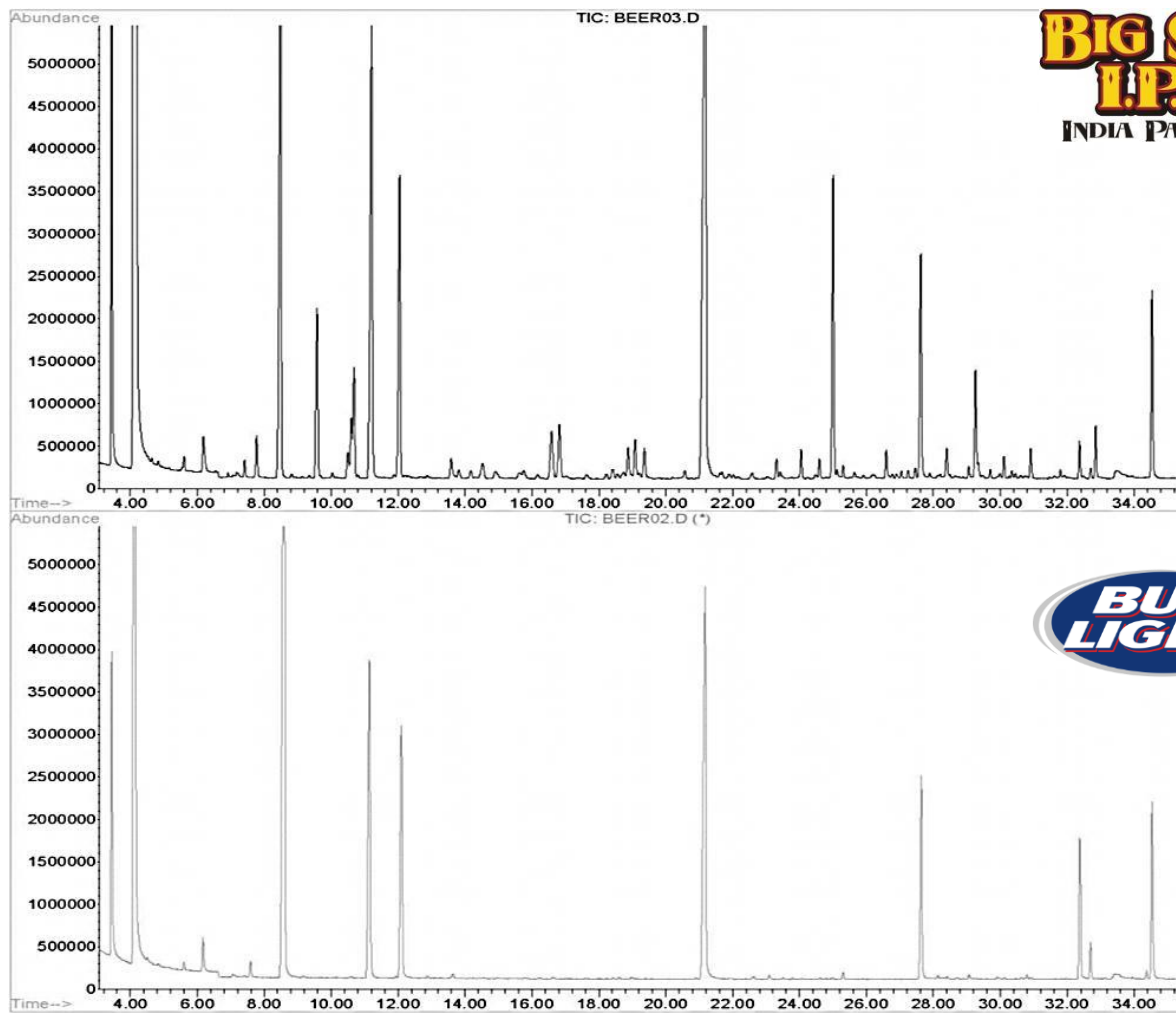
**Sample Preparation Method:** Solid phase microextraction

**Identification + quantification:** Gas Chromatography & Mass Spectrometry





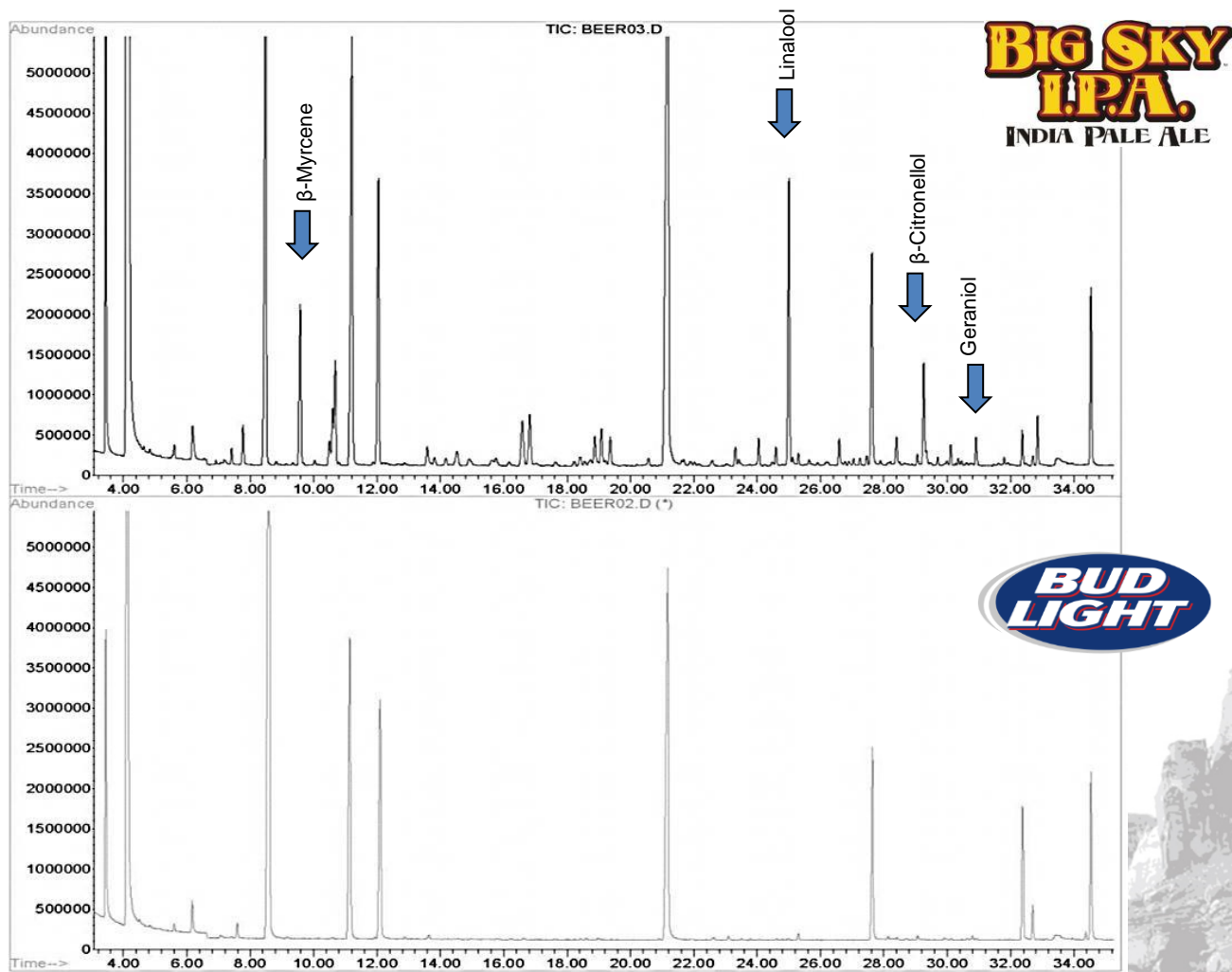
# HS-SPME GC-MS of Big Sky IPA and Bud Light







# HS-SPME GC-MS of Big Sky IPA and Bud Light





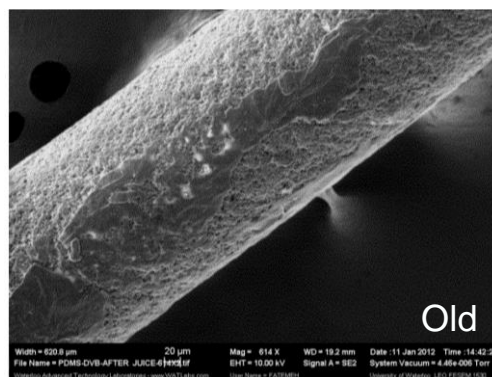
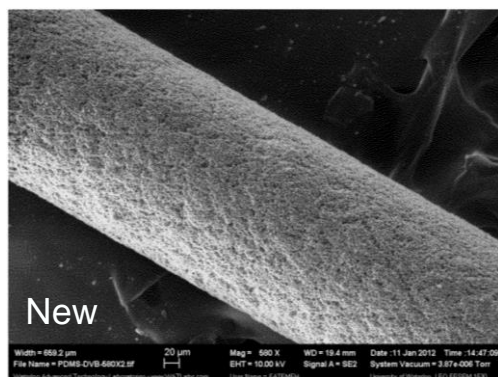
# Potential Pitfalls with SPME GC-MS Hop Aroma Analysis

**Method is not yet validated via an international ring study**

Solid phase microextraction

## Operational headaches with SPME

- Reproducibility issues
- Finite Fiber life
- Competitive absorption onto the fiber



Thanks for your attention

Any questions?



Brewing Science

