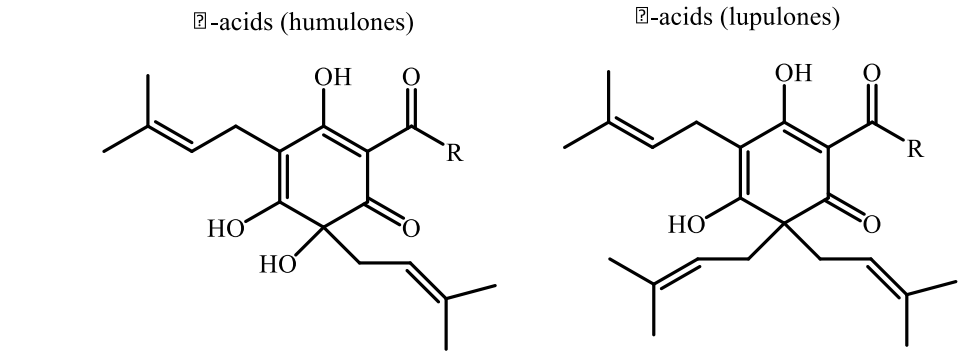
Analysis of α- and β-Acids in Hops by HPLC. Part 1

Reading Adapted from Travis M. Danenhower, Leyna J. Force, Kenneth J. Petersen, and Thomas A. Betts*: *Journal of Chemical Education*, 85(7), 2008, 954-955. Analysis by the EBC 7.7 test method is from the *Analytica-EBC*, by European Brewers Convention, 2005.

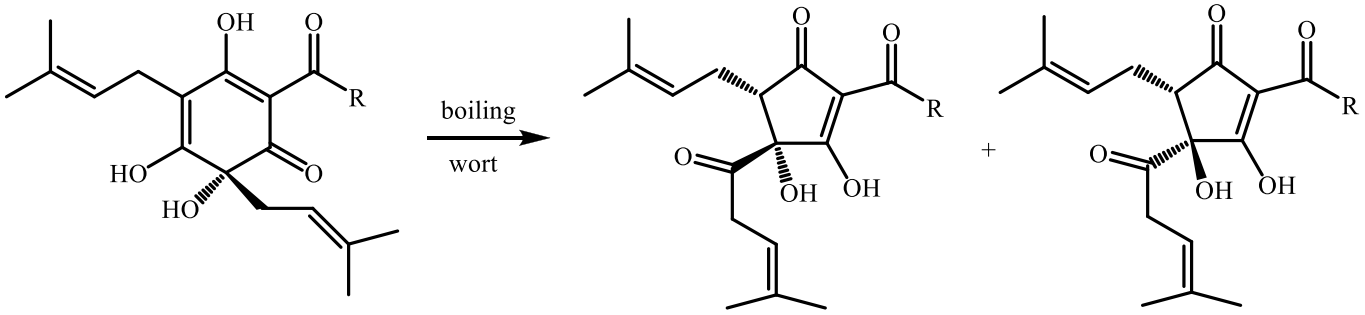
Introduction

Hops are used to impart bitter flavor and aroma to beer. Within the cones of the female hop plant are substances generally described as α- and β-acids. The predominant α-acids (humulone, cohumulone, and adhumulone) are precursors to the bitter compounds that balance the sweetness of the malted barley. The corresponding β-acids (lupulone, colupulone, and adlupulone) contribute significantly less to overall bitterness than α-acids.



R = $CH_2CH(CH_3)_2$	humulone	lupulone
$CH(CH_3)_2$	cohumulone	colupulone
$CH(CH_3)CH_2CH_3$	adhumulone	adlupulone

Hops are often added as the mashed malted barely (or malt extract) is boiled in water. During this boiling process the modestly bitter α-acids undergo thermal isomerization to form extremely bitter iso-α-acids. The more soluble and stable iso-α-acids contribute the greatest extent to the bitter flavor of beer. Beta-acids are oxidized during the boil, rather than isomerized. These oxidation products of the β-acids do influence taste and aroma, but to a much lesser extent than the α-acids.



humulones *cis*-isohumulones *trans*-isohumulones

It is important for brewers not only to understand the character of different hop varieties (Cascade, Hallertauer, Fuggles, Saaz, etc.) add to brewed products, but also to predict the ultimate bitterness of a batch of beer. The bitterness of a brew depends primarily on the concentration of α-acids contained in the hops, the amount of hops

used, and the length of time the hops are boiled. In this experiment we will use high performance liquid chromatography (HPLC) to determine the α- and β-acid concentrations in hop samples. External standards will be prepared from an extract commonly used in the brewing industry. Known as the International Calibration Extract, or ICE-3, it is from a specific strain of hop called Hallertau Perle hop. Another standard will be used to measure the amount of iso-α-acids, known as the International Calibration Standard, or ICS-I3.

Prelab

Along with the usual **Name**, **Title**, **Purpose** and **Outline**, list the HPLC conditions below, and prepare a table to record the percent composition of the ICE-3 and the ICS-I3 standards (this data can found at the class website in the Laboratory folder).

Materials

HPLC System

Reverse-Phase Column:	4.6 X 150 mm, (3.5 μm, 80 Å) Agilent Zorbax Extend C-18 set at 40°C
Mobile Phase:	85% Methanol / 15% water, acidified with 0.1% v/v trifluoroacetic acid (Filtered and degassed)
Flow Rate:	1.4 mL/min.
UV Detector:	326 nm (α-acids) and 270 nm (for iso-α-acids)
Injection. Volume:	50 μL
Extraction Solvent:	85% Methanol / 15% water, acidified with 0.1% v/v trifluoroacetic acid
Other:	0.45 μm nylon syringe filters, 50 mL volumetric flasks (5), 25 mL volumetric flask, beakers (100 mL, 250 mL), magnetic stirrer, medium porosity filter paper (Whatman no. 4), vacuum filtration system.

Hazard Warning

Methanol is flammable and toxic! Use caution when dispensing these solvents, and keep them away from ignition sources. Clean up any spills immediately. Methanolic HPLC wastes and diethyl ether extras should be discarded in the Non-Halogenated Organic Liquid waste container. Any aqueous wastes from the extraction should be discarded in the Aqueous Wastes container.

Hops and hops extracts contain compounds that, when in concentrated form, are know to be irritating to the skin and mucous membranes. Use gloves when handling hops and wash your hands thoroughly with soap and water after the experiment.

Procedure

You will be assigned a sample of one of the following commercially available hops: Northern Brewer, Czech Saaz, Super Galena, Cascade, or Columbus, or Tettnang. Complete the following steps:

1. Crush hops with a mortar and pestle. Accurately weigh a 0.25 g sample of hops and place hops in a 50-mL Erlenmeyer flask. (accurrately weigh a second sample of the same hops for Part 2)

2. Add approximately 20 mL of the Extraction Solvent. Sonicate the sample for 5 minutes, then stir the solution for 15 minutes at room temperature using a magnetic stir bar.

3. Vacuum filter the entire mixture using a Hirsh funnel with medium porosity filters paper (Whatman, No. 4), and collect *all* of the filtrate. Be sure to *quantitatively* transfer all of the solids and solution from the flask into the funnel, by rinsing with two to three times with 2-3 mL each of the Extraction Solvent.

4. Using a polyethylene disposable pipette, rinse the solid on the filter paper with approximately 10 mL of extraction solvent in 2-mL increments.

5. Quantitatively transfer the filtrate to a 50-mL volumetric flask and dilute to the mark using extraction solvent (to the bottom of the meniscus).

6. Using a 0.45 μm nylon syringe filter, add ~1.5 mL of filtered solution from the previous step directly into an autosampler vial.

7. Inject the filtered samples into the HPLC according to the instructions provided by your laboratory instructor. Obtain the resultant chromatogram for each sample.

To Complete the Experiment (partial report)

Data Analysis

A set of 5 calibration standards were prepared and run on the instrument using the HPLC conditions from above. They were prepared by diluting a 50.00 mL stock solution a known amount of the ICE-3 standard mixture of α- and β-acids and of the ICS-I3 standard mixture of the iso-α-acids. Your instructor will provide you with the actual amounts used for the analysis. Five different concentrations of the mixtures were prepared by adding 0.500 mL, 0.750 mL, 1.50 mL, 3.00 mL, and then 6.00 mL of the stock solution to a 10.00 mL volumetric flask and then diluting to the mark using the extraction solvent.

Using the percent composition information provided for the ICE-3 standard, determine the concentrations of all of α-acids in mg/mL for each of the calibration standards (cohumulone, humulone + adhumulone). Also, in the same way, calculate the total concentration of the iso-α-acids in each calibration standards. The weight percent of the combined iso-α-acids is provided in the information on the ICS-I3 standard.

Use the peak areas from the data for the calibration standards to create a table that shows peak retention time, peak area, and concentration. There are four major peaks in the chromatogram of the standard, which are due to six α- and β-acids. Under these separation conditions the α-acids elute first, then the β-acids. The identity of these peaks are shown in the ICE-3 information on-line. Using the peak areas from the standard chromatograms, create four calibration curves (peak area v. concentration in mg/mL):
1. cohumulone,
2. adhumulone + humulone
3. isocohumulone+isoadhumulone+isohumuone (all three are integrated together).

An example of one of the curves is inluded.

Calibration Data:

Calibration standard	cohumulone		humulone + adhumulone	
	C (mg/mL)	Peak area	C (mg/mL)	Peak area
1				

Calibration Results for α- and β-acids:

Calibration curve	cohumulone	humulone + adhumulone
slope		
intercept		

Similarly, prepare a table for the data for peak area and concentration on the iso-α-acids. Also, show a table that reports the final slope and intercept (note there is only on peak area for all of the iso-α-acids). You will use this data for Part 2 of this exeriment.

From the chromatogram of the your hop sample determine the concentrations (or combined concentrations for adhumulone + humulone and adlupulone + lupulone) in mg/mL of the α- and β-acids in the hop extracts using their respective calibration curves. Then calculate the weight % of the α- and β-acids in the original hops samples. Add the concentrations of the α-acids (cohumulone plus adhumulone + humulone) and β-acids (colupulone plus adlupulone + lupulone), and report the total α-acid and total β-acid concentrations (% w/w) in the hop samples. Finally, include the reported α-acid content from the label of the hops that you analyzed. compare the two methods of extraction – which method gave a higher value for the α-acid concentration?

Results:

Sample	cohumulone % w/w	humulone + adhumulone % w/w	Total α-acids % w/w	Reported α-acid % w/w
1				
2