

HPLC Analysis of α - and β -Acids in Hops

Travis M. Danenhower, Leyna J. Force, Kenneth J. Petersen, and Thomas A. Betts*

Department of Physical Sciences, Kutztown University of PA, Kutztown, PA 19530-0730; *betts@kutztown.edu

Gary A. Baker

Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6110

Early in brewing history, a variety of herbs and spices (such as coriander, rosemary, yarrow, and bog myrtle) were used to flavor beer (1). It is evident, from the Bavarian Purity Law of 1516, that a major shift in beer flavoring occurred around the middle of the second millennium. This law declared that only three ingredients could be used to brew beer: barley, water, and hops (1), thus eliminating other spices from German beer. (The importance of yeast had not yet been uncovered.)

A small quantity of hops has a substantial impact on the characteristics of a particular brew. Not only do hops contribute to the aroma and bitterness of beer that balance the sweetness of

the residual sugars from the malted barley, but they also stabilize beer foam, improve colloidal stability, and help preserve the wort (unfermented beer) (1). Under certain conditions, hops can also cause an unfavorable, "lightstruck" or skunky flavor (2–4).

The components that ultimately impart bitter flavor to beer, known as α - and β -acids, are produced in the cones of the female hop plant (*Humulus lupulus*). The predominant α -acids (humulone, cohumulone, and adhumulone; Figure 1) obtained directly from hop cones taste only slightly bitter (5). However, as hops are boiled in wort, the α -acids undergo thermal isomerization (Figure 2) to produce iso- α -acids that are intensely bitter (6). Beta-acids (lupulone, colupulone and adlupulone; Figure 1) are oxidized during the boil, rather than isomerized (7). These oxidation products of the β -acids influence taste and aroma, but to a much lesser extent than α -acids, and are often considered to unfavorably impact flavor. Therefore, it is important that brewers know the initial α - and β -acid concentrations of hops, as well as the extent of α -acid isomerization, so they may predict the ultimate bitterness of a particular brew.

There are many interesting high-performance liquid chromatography (HPLC) experiments suitable for undergraduate chemistry courses that involve the analysis of consumer and food products such as analgesics (8–11), asthma medication (12), sunscreens (13), cosmetic cream and jam (14), infant formula (15), collard greens (16), spinach (17), mussels (18), hot peppers (19–21), vanilla flavoring (22–24), milk (25), sports drinks (26), coffee (27), soda (28–33), citrus juices (28, 34, 35), and wine (28, 36–38). The experiment described here, the quantitation of α - and β -acids in hops, provides instructors with an interesting, rugged, time-efficient separation, that may be executed with straight-forward sample preparation and modest HPLC equipment.

Many published chromatographic methods for the analysis of α - and β -acids in hops require 35 to 60 minutes (5, 39–41), making these methods difficult to implement in a typical three-hour laboratory. By modifying one of these isocratic methods (5) we were able to achieve resolution adequate for quantitative analysis in a chromatographic run of less than 7 minutes. It is appropriate for inclusion in an undergraduate analytical chemistry or instrumental course, and would nicely complement lectures related to the chemistry and biochemistry of brewing (4, 42–44). Optional follow-up laboratory exercises provide students with opportunities to assess various aspects of quality assurance or method validation.

Experimental Procedure

Materials

An international calibration extract (ICE-2) for α - and β -acids in hops can be obtained from the American Society of Brewing Chemists (ASBC, St. Paul, MN). Both the extraction solvent and mobile phase are composed of 85% (v/v) HPLC-grade methanol (Fisher) and 15% (v/v) distilled, deionized

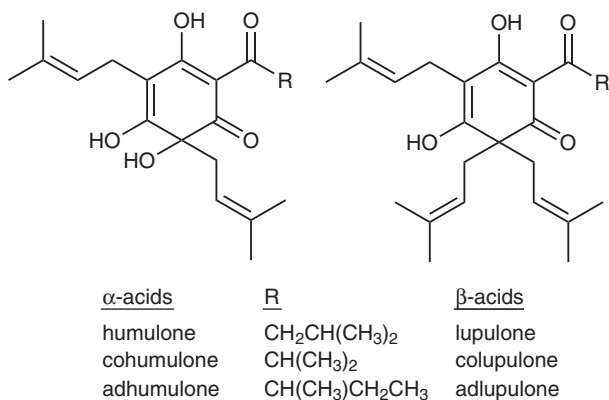


Figure 1. Structures of predominant α - and β -acids present in hops.

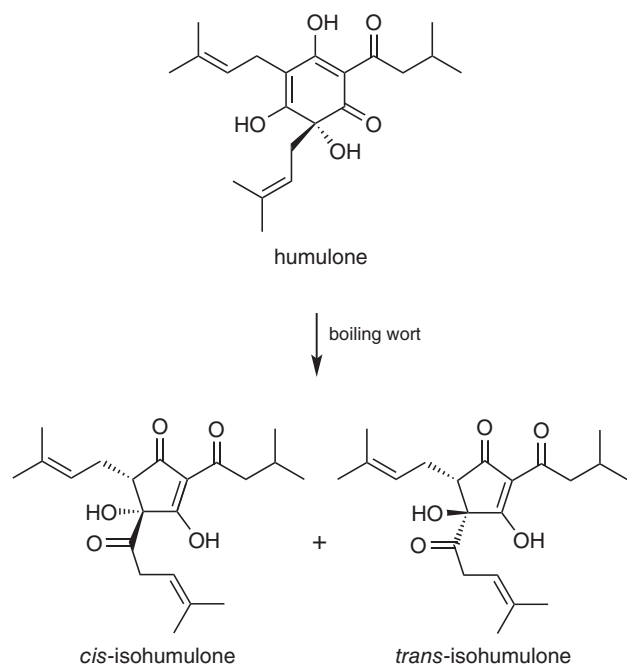


Figure 2. Thermal isomerization of humulone.

water. This mixture is acidified with 0.025% (v/v) formic acid (Fisher). We purchased a variety of hop pellets from Reading Carbonics, Inc. (Reading, PA), and locally grown loose Cascade hops were a generous gift from Daniel J. Ryder. All hops and the ICE-2 standard were stored at $-20\text{ }^{\circ}\text{C}$ in the dark as soon as they were received.

Standard and Sample Preparation

Working standards of α - and β -acids are prepared by dissolving an aliquot of the ICE-2 hop extract in extraction solvent, filtering, then diluting with extraction solvent to achieve cohumulone concentrations in the range of approximately 0.01 to 0.50 mg/mL. Hop samples are crushed and stirred in extraction solvent for one hour, then vacuum-filtered, diluted, and syringe-filtered prior to HPLC injection.

HPLC Analysis

An Agilent 1100 HPLC system was used for routine analysis, but this isocratic method is capable of being executed on virtually any functional HPLC with a UV detector. The chromatographic conditions are included in the caption to Figure 3. The detector wavelength was set at the absorbance maximum of the diluted ICE-2 standard (326 nm), but these compounds also absorb sufficiently at 254 nm. Alternate C-18 columns require slightly different mobile phase compositions to minimize chromatographic run time while maintaining acceptable resolution. Results and conditions for alternate columns are included in the online supplement associated with this article.

Hazards

Methanol is flammable and toxic if ingested. Formic acid is moderately flammable and toxic but is highly destructive to skin and eyes. The extraction solvent and mobile phase should be prepared in the hood away from ignition sources using appropriate precautions. Methanolic HPLC waste must be disposed of in accordance with federal, state, and local environmental regulations.

Results and Discussion

The chromatogram of the ICE-2 standard (Figure 3) shows four predominant peaks for six α - and β -acids. Cohumulone and colupulone elute at 2.94 and 4.50 min, respectively. The peak at 3.43 min is due to the coelution of humulone and adhumulone, while the peak at 5.45 min is due to the coelution of lupulone and adlupulone. Because the purpose of this analysis is to quantify the total α -acid and total β -acid content of the hop samples, it is not necessary to resolve individual humulones and lupulones. In fact, the documentation supplied with the ASBC ICE-2 standard provides combined concentrations for humulone + adhumulone, as well as lupulone + adlupulone. The reader is referred to the work of others if they wish to resolve all six compounds (40).

Students' calibration curves for the four resolved humulone and lupulone peaks clearly demonstrate that peak area is linearly related to concentration. The squares of the correlation coefficients for the four calibration curves typically ranged from 0.990 to 0.999 with residuals randomly distributed about a linear model. The accuracy of this method was assessed by spike recoveries that ranged from 97.7 to 101%, and the 1-hour extraction was determined to be 99% efficient.

Over the past two years, analytical chemistry students have analyzed a variety of hop samples for α - and β -acid content (Table 1). The most striking result of these analyses is the discrepancy between the concentration of α -acid printed on the label and the results of our HPLC analyses.

It is well known that α - and β -acids oxidize in the presence of light, heat, and oxygen (7, 45, 46) and can continue to degrade even under proper storage conditions. Also, some varieties of hops are more susceptible to degradation than others (46). The age and storage conditions of our hops were unknown prior to our acquisition and are likely causes for the discrepancies between package labels and the results of HPLC.

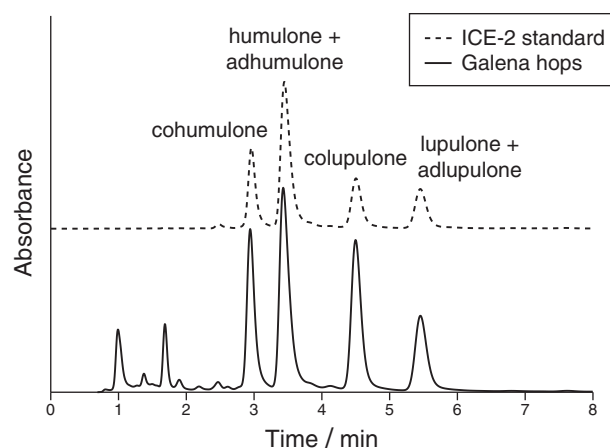


Figure 3. HPLC separation of ASBC international calibration extract (ICE-2) compared to an extract of Galena hops. A 4.0×125 mm Hypersil ODS column with $5\text{-}\mu\text{m}$ particle diameter was used with an 85:15 v/v methanol-water mobile phase acidified with 0.025% v/v formic acid flowing at 1.0 mL/min. UV detection was at 326 nm.

Table 1. Alpha- and Beta-Acid Content of a Variety of Hops

Hops Sample	α -Acid Conc		β -Acid Conc
	Listed on Label/ %w/w	By HPLC/ % w/w	By HPLC/ % w/w
Loose Cascade	—	1.3	1.8
Fuggle	3.3	2.6	2.3
Hallertau	3.5	1.9	1.9
Kent Goldings	4.1	2.9	1.8
Willamette	4.1	2.5	2.9
Styrian Goldings	4.9	3.6	2.5
Saaz	5.0	2.5	2.9
Cascade	5.6	4.8	5.3
Perle	6.3	4.5	2.9
Progress	8.0	2.4	1.0
Target	8.4	4.0	1.9
Target	10.4	8.6	4.2
Nugget	12.2	9.0	3.4
Galena	13.0	9.3	6.8
Columbia	16.1	12.6	4.1

NOTE: All hops samples were in pellet form with the exception of the loose cascade hops.

If time and instrument resources are available for a follow-up HPLC experience, several extensions to this analysis could be assigned that would allow students to apply their quantitative HPLC skills to concepts of quality assurance or method validation (47). For example, students could be asked to determine the efficiency of the hop pellet extraction, assess the accuracy of the method using spike recovery, use analysis of variance (ANOVA) to distinguish between the standard deviation of sampling versus the measurement, or determine the limits of detection and quantitation. We encourage our students to develop their own experimental plan by providing minimal instruction for these tasks.

Conclusions

The application of HPLC to the analysis of hops provides another opportunity for instructors to tailor experiments to student interest in order to foster excitement in the laboratory. Many of our students looked forward to executing this laboratory experiment after they saw the laboratory schedule. This exercise can be implemented with minimal HPLC equipment (isocratic, fixed wavelength detection) and a standard C-18 column. Several extensions to this laboratory exercise are suggested to allow students to apply what they have learned about quantitative HPLC to concepts of method assessment and validation. While the first portion of this experiment provides a fairly standard introduction to HPLC analysis, our students found that the extensions significantly challenged their ability to implement their recently acquired HPLC skills within their own experimental design.

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