APPLICATION NOTE

UHPLC

Authors

Njies Pedjie

PerkinElmer, Inc.
Shelton, CT USA

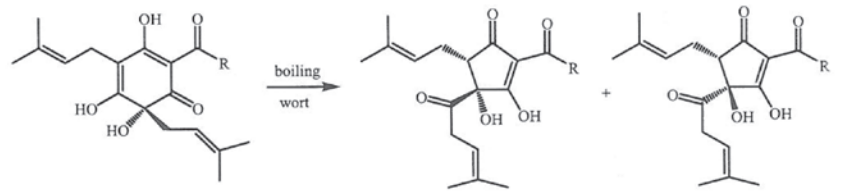
Determination of α-acids in Hops and Beers

Introduction

Hops are crucial in beer brewing. They are added after the malting of the grains and provide beers with their recognizable bitter taste and aroma. The widespread use of hops in beer dates back to the sixteenth century. However, as early as in the eleventh century it was used as a natural preservative in central Europe (today Germany); the outcome was not only a well preserved beer, but a beer with a distinctive smell and taste.

Hops come from a cone-like plant called *Humulus lupulus* with luplin gland that contains resin and oils. The resins contain a number of α-acids that impart the bitter taste to most beers; the oils in large part give beers their aroma.

One essential aspect of the quality control in beer brewing is making sure that the type and amount of α-acids are the same from batch to batch, and that their transformation into the bitter iso-α-acids during the brewing process gives individual brand its recognizable taste consistently (Figure 1). To that end, in breweries around the world, α-acids in hops and beers are constantly monitored. This application note presents a straightforward method to determine the type and amount of α-acids in pellets from five hops varieties. An American IPA beer is analyzed to confirm the presence of isomerized α-acids.



ICE 3 (α-acids)
CH(CH3)2 cohumulone
CH2CH(CH3)2 humulone
CH2 (CH3) C2H5 adhumulone

cis-iso-α-acids
cis-cohumulone
cis-humulone
cis-adhumulone

ICS- I3 (*trans*-iso-α-acids)
trans-cohumulone
trans-humulone
trans-adhumulone

Figure 1. Isomerization of hop α-acids to iso-α-acids during brewing.

PerkinElmer®
For the Better

Experimental

A stock solution of 7.3 mg/mL of ICE 3(α-acids), and another of 1.4 mg/mL of ICS-I3 (isomerized α-acids) were prepared by transferring the appropriate net weights into a 10 mL vol. flask, methanol was added to volume followed by 10 min. sonication. From the stock solutions five levels of working standard with concentrations of α-acids ranging from 23 µg/mL to 440 µg/mL (23, 55, 110, 220, 440 µg/mL) were prepared by spiking appropriate volumes of the stock solutions into five 10 mL volumetric flasks followed by dilution to volume with sample solvent. The two lower levels were direct 1:1 dilution from the preceding level. Repeatability was evaluated with seven injections of the 440 µg/mL working standard.

About 0.2 g of each of the hop pellets (German Spalt, American Cascade, American Summit, English Fuggle, New Zealand Nelson Sauvin) were transferred into individual 10 mL volumetric flasks. The flasks were half filled with the sample solvent and let to soak for four hours while vortexing every hour. The preparation was sonicated for 15 min. in cold water then brought to volume, mixed well and centrifuged at 7000 RPM for 10 min. Each supernatant was transferred into a 25 mL vol. flask and set aside. Ten mL of sample solvent was added on each remaining precipitate followed by a vigorous vortexing for about two minutes. This latter preparation was also centrifuged for 10 min. at 7000 RPM, the supernatant was collected and transferred into the corresponding 25 mL vol. flask previously set aside. The flask was brought to volume and filtered through a 0.2 µm nylon filter prior to testing.

The beer samples were prepared by Liquid-liquid extraction (LLE). 0.5 mL of phosphoric acid and 10 mL of trimethylpentane were added to 10 mL of degassed American IPA beer. After a minute of vigorous vortexing and 15 minutes of sonication in cold water, the preparation was centrifuged for five min. at 2000 RPM. 5.0 mL of the supernatant was collected and evaporated using a nitrogen evaporator; the residue was dissolved in 2.0 mL of solvent.

The accuracy of the method was evaluated by spiking 10 mL of a low hops beer previously degassed with a volume of stock standards as to obtain levels of acids of 440 µg/mL. The same liquid-liquid extraction was applied, but in this instance, 4.0 mL of the supernatant was collected and evaporated using a nitrogen evaporator; the residues were dissolved in 4.0 mL of solvent. Similarly, a control was prepared. All samples were filtered through a 0.2 µm nylon filter prior to testing.

Chromatographic conditions

Autosampler:	Flexar™ FX UHPLC
Setting:	50 µL loop and 15 µL needle volume Partial loop injection mode
Injection:	4 µL; Flush solvent: 1:1 methanol/water
Flow:	1 mL/min
UV detector:	270 nm
Column:	Brownlee™ SPP C18, 100 x 3.5 mm, 2.7 µm at 40 ° C Cat. No. N9308410
Isocratic:	35% mobile phase A: 0.1% phosphoric acid, 0.2 mmol/L EDTA 2NA 65% mobile phase B: acetonitrile
Sample solvent:	8:2 methanol / 0.1% Trifluoroacetic acid (TFA) in water (HPLC grade solvent and ACS grade reagent)
Software:	Chromera™ version 3.0
Sampling rate:	5 pts/s

Results and Discussion

A PerkinElmer® Flexar FX-15 fitted with UV Detector was the platform used for this analysis. The separation was achieved using a Brownlee SPP C18, 100 x 3.5 mm, 2.7 µm column. Optimal flow rate was 1.0 mL/min with a modest back pressure of approximately 3500 PSI (241 bars) and the run time was five min. The repeatability of seven injections of the 440 µg/mL standard was excellent with %RSD values ranging from 0.7 to 1.5. Furthermore, the average %RSD across the five calibration levels were less than 2.0. Calibration curves exhibited excellent linearities with $r^2 \geq 0.999$. The LLE extraction technique was excellent with level of α-acid detected in the IPA beer and an average accuracy result of 95%. Results of the analysis of the hops pellets tested matched the label claims. Method performance including calibration curves, precisions, tailings and resolutions are presented in Figure 1 and Table 1. Representative chromatograms of the standard, American Cascade hops, New Zealand Nelson hops and American IPA beer solutions are shown in Figures 2, 3, 4, 5. Hops pellets assay results are in Table 2.

Table 1. **Method performance.**

α-acids	Repeatability (n = 7)	Resolution	Tailing	Accuracy
t-isocohumulone	0.8	–	1.5	99%
t-isohumulone	0.7	6.8	1.4	99%
t-isoadhumulone	1.1	2.5	1.0	90%
cohumulone	1.1	5.8	1.1	92%
humulone	1.5	8.1	1.1	96%
adhumulone	1.1	2.3	1.0	96%
Average	**1.1**	–	**1.2**	**95%**

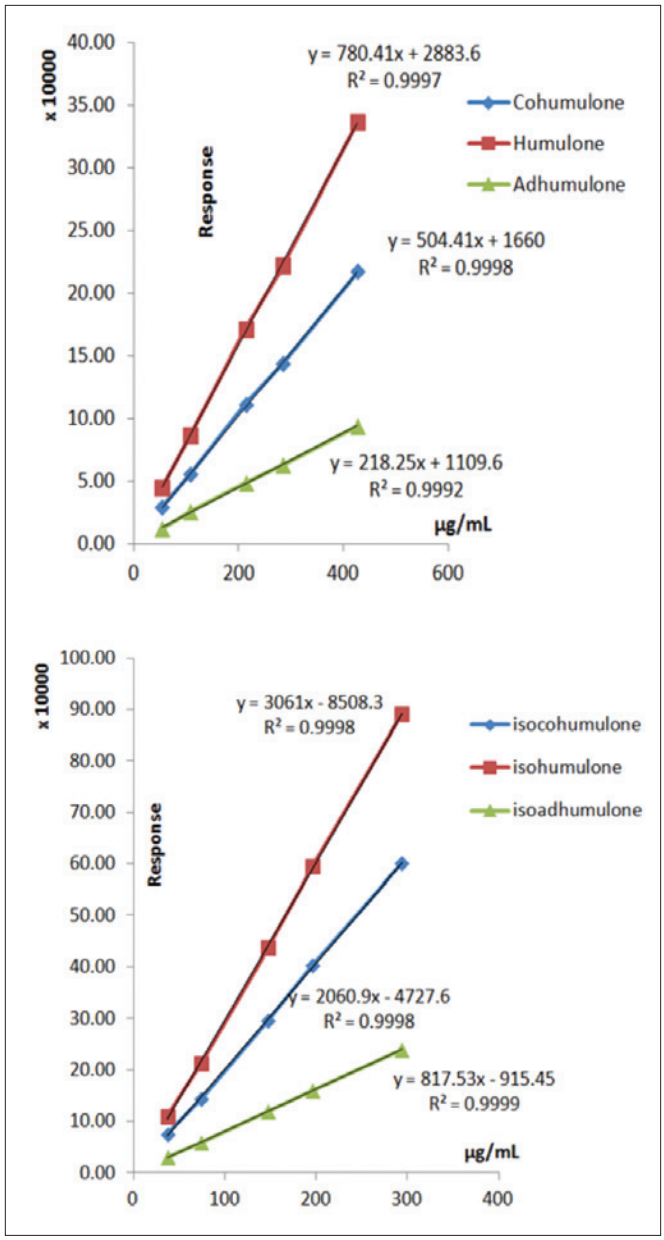


Figure 1. Calibration curves, regression and intercepts.

***Table 2.* Label claim and assay results.**

Species of Hops	Analysis results	α-acids label claim	% of label claim
German Spalt	2.3%	2.6%	87%
American Cascade	4.9%	5.5%	90%
American Summit	16.6%	16.8%	99%
English Fuggle	3.4%	4.2%	81%
New Zealand Nelson	11.6%	12.0%	97%
Average	–	–	**91%**

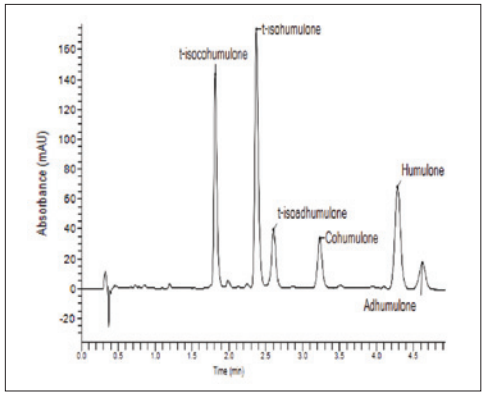


Figure 2. Chromatogram of a ECE 3, ICS-I3 standard 2.

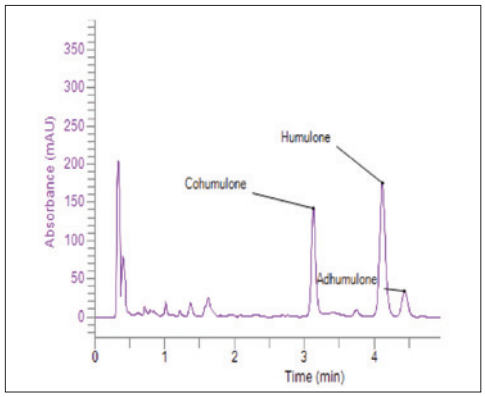


Figure 3. Chromatogram of American Cascade hops.

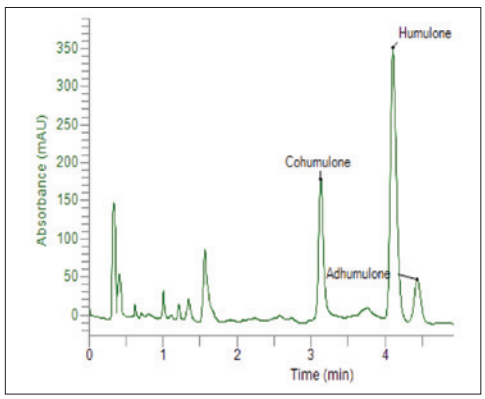


Figure 4. Chromatogram of New Zealand. Nelson hops.

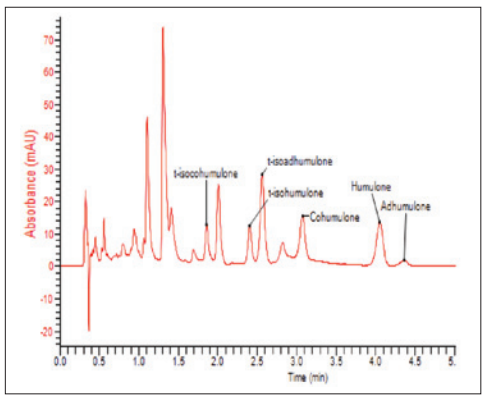


Figure 5. Chromatogram of American IPA beer.

Conclusion

In this analysis the six α-acids were well resolved in five minutes with resolutions between consecutive peaks ranging from 2.3 to 8.1. The method performance was outstanding with calibration correlation coefficient (r^2) not less than the cutoff of 0.999 and precisions with %RSDs ≤1.5. Peaks were sharp and symmetrical with tailing factor values ≤1.5. The extraction technique was very effective with an average recovery of 95%. Assay results showed levels of α-acids in hops matching the labels claims. The analysis the IPA beer confirmed the presence of isomerized α-acids.

PerkinElmer's FX-15 pump fitted with durable pistons and a highly robust autosampler are designed for great injection precision. The Brownlee superficially porous particle column with its innovative construction reduces the sample diffusion path resulting in fast separation, sharp peaks and a modest back pressure.

References

Enhance Quantitative Extraction and HPLC Determination of Hop and Beer Bitter acids. B. Jaskula, K. Goiris., G. De Rock, G. Alert, L. De Coonan: J. of The Institute of Brewing, 2007, Vol.113(4), 381-390.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

010784_01