APPLICATION NOTE

UHPLC

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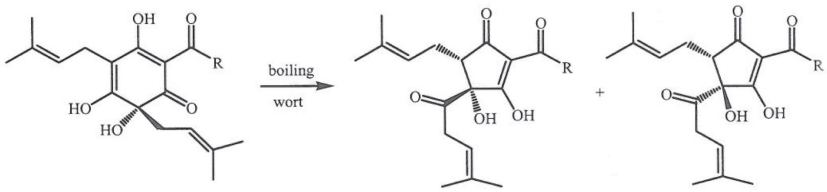
Determination of α-acids in Hops Using Third Party Software

Introduction

Hops are a major ingredient used in beer brewing. They preserve beer and provide it with its recognizable bitter taste and aroma. Hops come from a cone like plant called Humulus lupulus, which houses a lupulin gland containing resins 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.

In beer breweries around the world, one essential part of quality control is ensuring 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 consistently gives individual brands their recognizable taste (Fig. 1).

This application note presents a straightforward method to determine the type and amount of α-acids in pellets from three hops varieties.



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.

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Experimental

A stock solution of 7.0 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. of sonication. From the stock solutions, a 413 µg/mL working standard was prepared by dilution with sample solvent. Repeatability was evaluated with six injections of the working standard.

About 0.2 g of each of the hop pellets (American Cascade, American Summit, and New Zealand Nelson) were transferred into individual 10 mL volumetric flasks. The flasks were half filled with the sample solvent and left to soak for four hours while vortexing every hour. The preparation was sonicated for 15 min in cold water, and flasks were brought to volume with the sample solvent and centrifuged for 10 min at 7000 RPM. Each supernatant was transferred into a 25 mL vol. flask and set aside. 10 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 volumetric flask was brought to volume with sample solvent and samples were filtered through a 0.2 µm nylon filter prior to testing.

Chromatographic conditions

Platform:	Flexar™ FX-15UHPLC
Autosampler Setting:	20 µL loop and 250 µL Sample Syringe Volume Variable Loop injection mode Injection: 4 µL; Flush solvent: 1:1 methanol/water
Flow:	1.0 mL/min
UV detector:	270 nm
Column:	Brownlee™ SPP C18, 150 x 3.0 mm, 2.7 µm at 40 ° C cat# N9308411
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:	Agilent® EZChrom Elite™ Version 3.32
Sampling Rate:	5 pts/s

Results and Discussion

A PerkinElmer Flexar FX-15 fitted with a UV/VIS Detector was the platform used for this analysis. The separation was achieved using a Brownlee™ SPP C18, 150 x 3.0 mm, 2.7 µm column. The run time was eight min, the optimal flow rate was 1.0 mL/min with a modest back pressure of approximately 3500 PSI (241 bars). Method performance was excellent: repeatability % RSD values ranged from 0.4% to 0.8%, resolution between peaks ranged from 1.8 to 7.7, and peaks' tailing were less than 2.0. Assay results were 73%, 73% and 77% for the American Cascade, the American Summit and New Zealand Nelson, respectively. The α-acid in hops were lower than the label claims. Method performance results are shown in Table 1, Hops assay results are shown in Table 2. Representative chromatograms of the standard, American Cascade and American Summit solutions are shown in Figures 2, 3, 4.

Conclusion

In this analysis, the six α-acids were well resolved. The method was precise with %RSDs ≤ 0.8%. Assay results of hops pellets showed levels of α-acids 23% and 27% lower than the labels' claims. The column used was a Brownlee superficially porous particle column specifically designed to reduce the sample diffusion path and thereby deliver faster separation, sharp peaks, and modest back pressure. This analysis was done with the PerkinElmer FX -15 system reliably under the control of Agilent's® EZChrom Elite™ chromatography data system.

Table 1. Method Performance of RSD Standards.

α- acids	Repeatability % RSD (n=6)	Resolution	Tailing
t-isocohumulone	0.5	N/A	1.7
t-isohumulone	0.6	5.1	1.8
t-isoadhumulone	0.4	1.8	0.0
cohumulone	0.4	4.2	1.1
humulone	0.7	7.7	1.2
adhumulone	0.8	2.3	1.1
Average	0.55	----	----

Table 2. % Weight/Weight Analysis Results.

Species of Hops	% Results	% Label Claim	% of Claim
American Cascade	4.0	5.5	73
American Summit	12.2	16.8	73
New Zealand Nelson	9.2	12.0	77
Average	----	----	74%

References

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

Note: this application note is subject to change without prior notice.

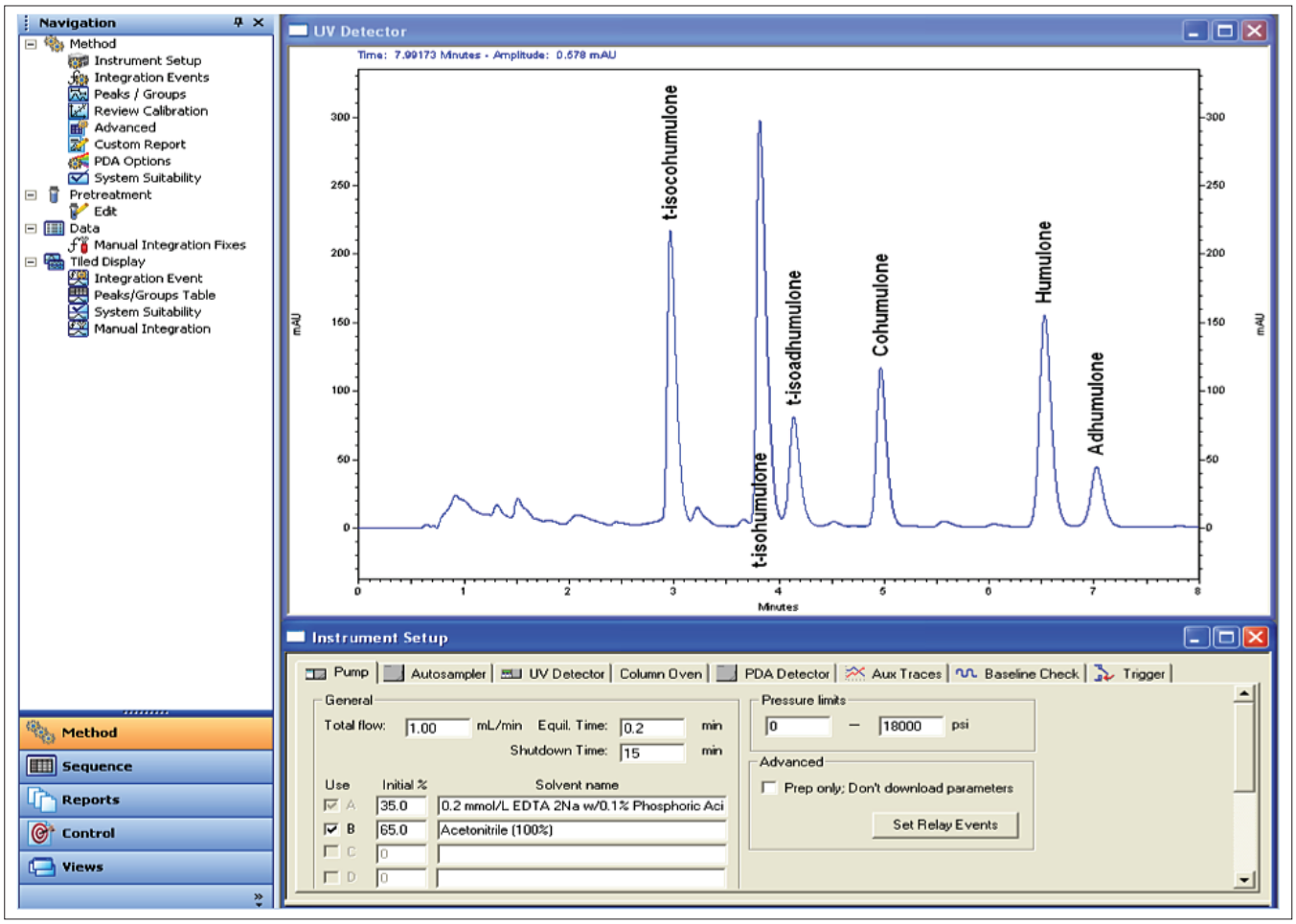

Figure 2. Chromatogram of a Standard With ICE 3 and ICS-I3.

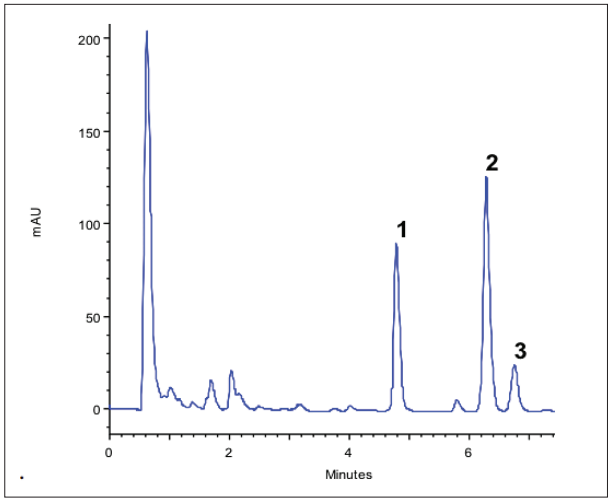

Figure 3. Chromatogram of the American Cascade Hops.
1. Cohumulone, 2. Humulone 3. Adhumulone

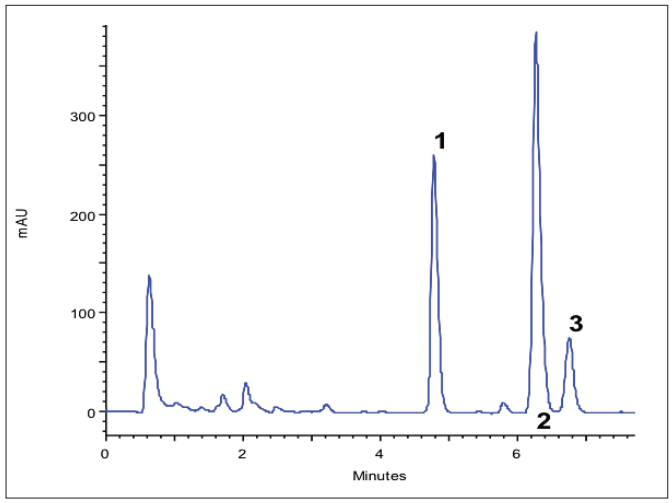

Figure 4. Chromatogram of the American Summit Hops.

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