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Assaying Hops and Beers with SFE followed by Flash LC or UHPLC

Phillip B. Liescheski & Jack E. Silver, Teledyne Isco, 4700 Superior, Lincoln, NE

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INTRODUCTION

The α- and β-acid profiles of hops and beer characterize their flavor and antibacterial properties, which are important in brewing and storing beers and ales. Hops have been assayed by organic liquid solvent extraction and followed by conventional reverse-phase HPLC. Recently, subcritical and supercritical carbon dioxide (i.e. Supercritical Fluid Extraction, a.k.a. SFE) has been used as an extracting solvent. In this poster, we explore the use on hops of SFE with carbon dioxide and Flash LC – a faster and simpler low-pressure liquid chromatography method. Using SFE with CO_2 as the solvent hops sample were extracted under different pressures. The extracts were assayed gravimetrically and qualitatively using TLC. Hop extracts from SFE, especially ICE-3, were further separated using Flash Liquid Chromatograph (LC). Several column media were explored under Flash LC: C18 (Reverse-Phase), Diol, Cyano, Silica and Alumina (Normal-Phase). Fractions were collected and their chemical purity-identity confirmed using TLC, Mass Spectrometry (MS), Infrared (FTIR) and wet chemistry, e.g. Methanolic Pb.

BACKGROUND

SFE is sample extraction without needing liquid solvents. Supercritical fluid is essentially a gas above its Critical Temperature, meaning: it can be compressed to near liquid density without condensing into a liquid phase. Due to its high density, it's a gas that behaves like a solvent. Being a gas, it diffuses faster through the sample matrix, thus shortening extraction time. SFE also reduces the need to work with large volumes of flammable liquids. Liquid samples, such as beer, can still be extracted by absorbing onto diatomite. SF CO_2 performs like hexanes. In this poster, SFE is used to extract hops which can be further separated into fractions using Flash LC.

Flash LC is user-friendly Column LC. Using a high-flow pump for the mobile phase, instead of gravity feed, reduces separation time from hours to minutes. The emphasis is more on isolating sample fractions (prep) than number crunching (analytical). In this poster, Flash is used to refine ICE-3, also a SFE hops extract, into better standards for exploring other LC stationary phases and better UHPLC method development, since ICE-3 is already well characterized.

EXPERIMENTAL

Exploring Hops under SFE: Using a Teledyne Isco SFX-210 extractor, 260-HP syringe pump and Air Product food-grade (with dip tube) CO_2, samples of Hopunion Warrior® hop (15.8% α- & 4.4% β-acids) pellets were extracted. With its bag closed, the pellets were further pulverized with a rubber mallet and used "as is". Four grams of hops were introduced into each of three 10-mL disposable SFE sample cartridges. Each sample was extracted for 30 minutes at 50°C and 2 mL/min flow but CO_2 at either 2000, 5000 or 7500 psi. After the heated restrictor (60°C), each extract was trapped in a few-mL hexane in a collection vial. The hexane was blown down to dryness with UHP N_2 gas. The sample cartridges were weighed before and after extraction to determine weight loss. The extract collection vials were also weighed before and after extraction to determine weight gain. Gravimetric results shown in the table 1 below.

The extracts were assayed qualitatively by TLC with C18 and Silica plates against the ICE-3 hops standard. CO_2 at 5000 psi appeared to be the optimal extraction condition that gave gravimetric results similar to the expected total of α- and β-acids percent weight. Obviously the extraction was incomplete at 2000 psi and 30 minutes. The TLC results for the 5000 and 7500 psi extracts appeared similar to the ICE-3 trace. The silica TLC, was similar to neutral lipid test, showed little significant lipids, perhaps mainly waxes. Main spot occurs near organic acid area. The 7500 psi extract also showed an additional spot on the C18 plate that did not migrate and fluoresced red under 360 nm UV, suggesting Chlorophyll A. These extracts need better analyses, such as HPLC or even UHPLC, but these higher performance techniques need a standard better than ICE-3.

CO_2 Press. (PSI)	% Weight Loss	Extract Wt. (g)	% Extract Wt.
2000	5.6	0.2024	5
5000	22.5	0.8451	23
7500	24.1	0.8082	24.1

Table 1: Gravimetric Results of Warrior® hops after SFE

EXPERIMENTAL

Fractionating ICE-3 under Reverse-Phase (C18) Flash LC: The ICE-3 hops standard, which was prepared under SFE with CO_2, was fractioned into its four resolvable components using a Teledyne Isco EZPrep UV LC unit and a RediSep 15-g Gold C18 (40um beads) column. The mobile phase was water, as solvent A (weaker), and methanol, as solvent B, both with 0.025% formic acid and a flow rate at 30 mL/min. The gradient started at 75% methanol in water and increased to 100% over 50 column volumes (CV, which is 13.5 mL for this column). The separation was performed four times and the four fractions are collected for each run. The sample was introduced into the column as a solid sample, that is 1%-loading of ICE-3 fixed onto diatomite. The final run was a diatomite blank, which detected no peaks. Fractions from different runs but like peaks were combined and brought to dryness. Each dried fraction was weighed and compared to the original ICE-3 sample weight loading.

The four peaks in figure 1 are assumed to be due to (first) Cohumulone, (second) Adhumulone and Humulone, (third) Colupulone, and (fourth) Adlupulone and Lupulone [Danenhower, *et. al.*, p. 954]. Gravimetric results shown in table 2 below. The expected % weight values were obtained from the jar label of the ICE-3 standard. The collected % weight values were lower but that was expected since fractions suspected to contain overlapping peaks were discarded to maintain purity. Samples of these fractions along with the ICE-3 were assayed by C18 TLC (figure 4), confirming the consistency of the separation. FTIR spectra, figure 3, of all fractions were similar, suggesting an enolic acid instead of the usual carboxylic fatty acid. These fractions may be useful as standards for the LC analyses.

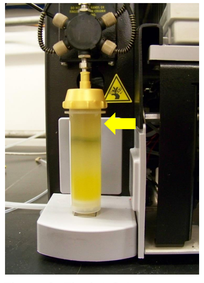

Figure 2: Flash LC Apparatus with Eluting Band

LC Peaks	Fraction Weight (g)	% Weight	ICE3 Jar Label Expected %
First	0.0873	13.56	13.88
Second	0.1818	28.25	30.76
Third	0.0623	9.68	13.44
Fourth	0.0576	8.95	10.84

Table 2: Gravimetric Results of ICE – 3 after Flash LC

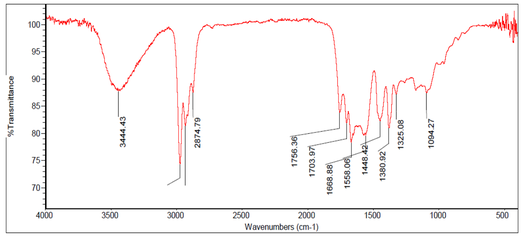

Figure 3: FTIR of Third Peak ICE – 3 Fraction: Enolic Acid

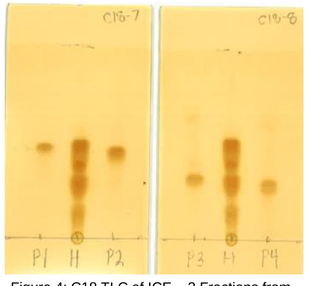

Figure 4: C18 TLC of ICE – 3 Fractions from Reverse – Phase C18 Flash LC

EXPERIMENTAL

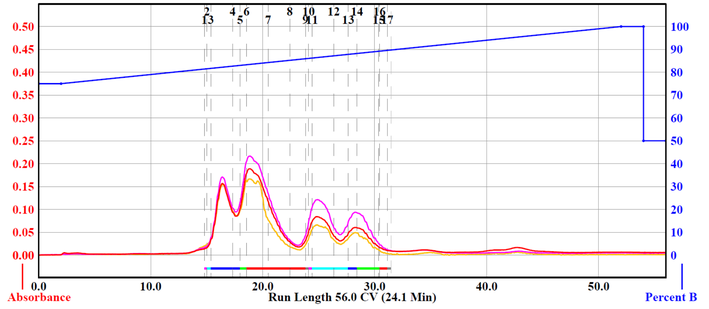

Figure 1: Reverse – Phase C18 Flash LC of ICE - 3

Exploring ICE-3 under other Normal-Phase Column Media:
The ICE-3 hops standard was also separated under Normal-Phase media: Diol, Cyano and Silica. During the preliminary TLC check, neutral Alumina plates showed serious streaking with the ICE-3, so it was not attempted under Flash LC. The EZPrep unit and RediSep Gold 15-g columns were used. The normal-phase mobile phase was cyclohexane, as solvent A (weaker) and 2-propanol with 0.5% Formic acid, as solvent B. At this point, separation of the acids did not appear to be as good as with C18. Using 4% lead acetate in methanol, the presence of α-acid was confirmed.

CONCLUSION

α- and β-acids were extracted from hops under mild conditions (50°C & 5000 psi) using CO_2. Higher-pressure SFE begins to extract other less desirable components, such as Chlorophyll [Neve, p. 47]. Low-pressure C18 Flash LC was capable of fractioning these acids into four components. Other normal-phase media shows promise in resolving the α- and β-acids into fractions also, but this is still a work in progress.

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