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Examination of Extraction Solvents to Improve Laboratory Efficiency and Reduce Solvent Use for the Analysis of Hop Products

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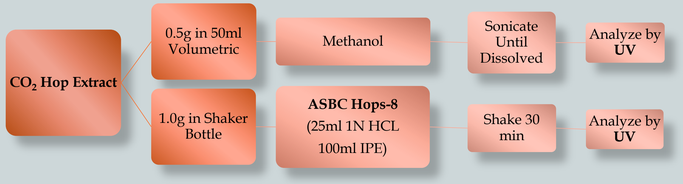
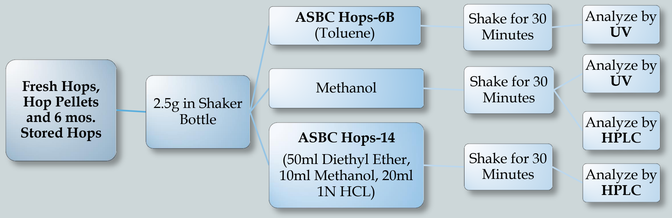
Authors: Cheryl Ermey, Patrick Jensen, Scott Garden – John I Haas Inc. Yakima, Washington

Introduction:
Analytical laboratories must provide accurate and timely results, however they also must be environmentally conscious, and provide a safe work environment. It is imperative that laboratories investigate methods to reduce solvent use, replace severe hazardous solvents with less hazardous solvents and to increase efficiency. This is an investigation into ASBC Ultraviolet Spectrophotometric (UV) and High Performance Liquid Chromatography (HPLC) hop analysis methods of hops, hop pellets and CO_2 extracts to reduce solvent use and increase laboratory efficiency by using a single solvent extraction protocol for ASBC hop methods. Currently the extraction solvent protocol for ASBC UV spectrophotometric methods is different from the extraction solvent protocols in HPLC methods.
Methanol is the extraction solvent used in HPLC analysis of CO_2 hop extracts, extraction solvent for HPLC standards. In this study we explored the use of a common methanol solvent protocol for the preparation of cone hops, hop pellets, and CO_2 hop extracts samples for UV spectrophotometric and HPLC analysis of their bitter hop acids components.

Methods and Materials:
CO_2 Hop Extracts: 52 CO_2 hop extracts were analyzed by UV spectrophotometry. The samples were extracted using two different extraction procedures.



Hops and Hop Pellets: 48 freshly harvested hops, 90 hop pellet samples and 89 hops stored for 6 months were analyzed by UV spectrophotometry and HPLC. The samples were extracted using three different extraction procedures.



The UV analysis procedure and HPLC analysis after extraction was exactly as written in ASBC methods Hops-6B, Hops-8 and Hops-14. All samples were analyzed using a Perkin Elmer Lambda 35 Spectrometer and an Agilent Technologies 1200 series HPLC. The standards used for comparison were made from the ASBC ICE-3 hop extract of known α- and β-acid content. After analysis the data was compared statistically using paired sample comparison student t test within 95% confidence.

Table 1: Paired Sample Comparison of ASBC Extraction Protocols vs. Methanol Extraction Protocol for UV and HPLC Analysis

Descriptive Statistics (Comparison of Methanol Extraction Protocol to ASBC Extraction Protocol)	CO2 Extracts		Fresh Hops			6 Month Stored Hops			Hop Pellets			Hops and Hop Pellets	
	α-Acids by **UV**	β-Acids by **UV**	α-Acids by **UV**	β-Acids by **UV**	HSI	α-Acids by **UV**	β-Acids by **UV**	HSI	α-Acids by **UV**	β-Acids by **UV**	HSI	α-Acids by **HPLC**	β-Acids by **HPLC**
Mean Difference	0.25	0.03	0.24	-0.55	-0.06	-0.01	-0.59	-4.93	-0.12	-0.65	-0.05	0.46	0.08
Standard Deviation	0.99	0.47	0.31	0.30	0.02	0.34	0.23	2.24	0.36	0.25	0.02	0.39	0.19
Variance	0.99	0.22	0.10	0.09	0.00	0.12	0.05	5.02	0.13	0.06	0.00	0.15	0.04
Observations	52	52	48	48	48	89	89	89	90	90	90	98	98
Degrees of Freedom	51	51	47	47	47	88	88	88	89	89	89	97	97
Critical T value Two-Tailed	2.01	2.01	2.01	2.01	2.01	1.99	1.99	1.99	1.99	1.99	1.99	1.98	1.98
t Calculated	1.84	0.43	5.23	12.67	18.58	0.26	23.70	20.78	3.22	25.19	24.37	11.53	4.05
Statistically the Same	Yes	Yes	No	No	No	Yes	No	No	No	No	No	No	No

Figure 1: Relative Percent Difference in α-Acid Analysis by UV for CO_2 Hop Extracts

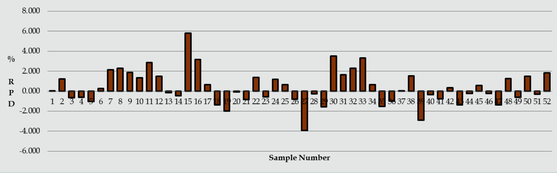
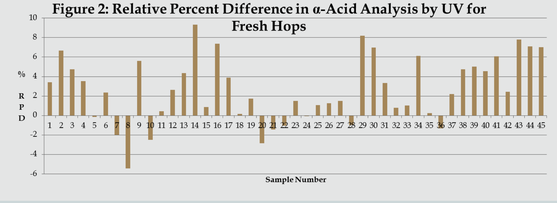


Figure 2: Relative Percent Difference in α-Acid Analysis by UV for Fresh Hops



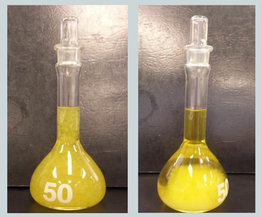


Photo 1 & 2: To the right are photos showing the turbidity/haziness of CO_2 hop extract in methanol when mixed and when allowed to settle out.

Figure 3: Relative Percent Difference in α-Acid Analysis by UV for Hops Stored for 6 Months

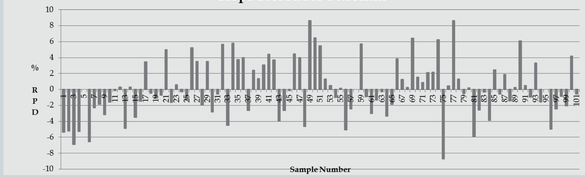


Figure 4: Relative Percent Difference in α-Acid by UV for Hop Pellets

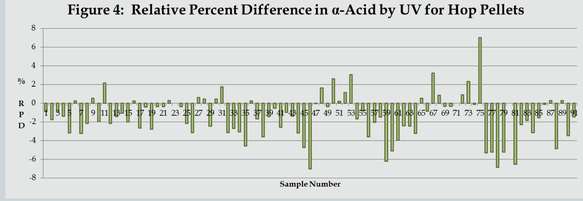
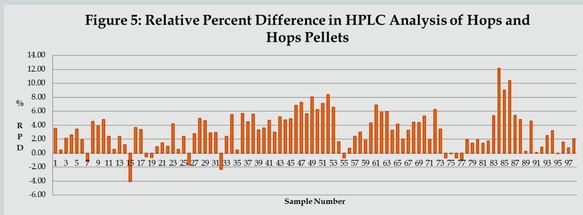


Figure 5: Relative Percent Difference in HPLC Analysis of Hops and Hops Pellets



Results and Discussion:
UV analysis α-acid and β-acid content in CO2 hop extracts showed no statistical difference between ASBC Hops-8 extraction protocol vs. methanol (Table 1). Figure 1 shows the relative percent difference (%RPD) between extraction protocols for α-acid analysis in each sample. It was observed that a turbid/hazy solution was produced when extracting CO2 hop extracts with methanol (Photo 1 and 2), however this did not affect the analysis results for α-acid and β-acid.

UV analysis of α-acid, β-acid and HSI content in freshly harvested hops, hops stored for six months and hops pellets showed a methanol extraction was statistically different to the ASBC Hops-6B extraction protocol (Table 1). It was observed that for hops stored for 6 months the two extraction protocols were statistically equivalent with respect to α-acid analysis only. Figures 2, 3 and 4 show the % RPD for α-acid analysis for each sample.

HPLC analysis of α-acid and β-acid content in hops and hop pellets showed a statistical difference in extraction protocols (Figure 5 and Table 1) when comparing ASBC Hops-14 to methanol. The ASBC Hops-14 extraction protocol was significantly higher than the methanol extraction.

The study further compared HPLC results for extraction protocol Hops-14 (Diethyl Ether) vs Hops-6B (Toluene), since the methanol extraction protocol did not produce equivalent results. The ASBC Hops-14 extraction protocol proved to have a statistically higher result for the analysis of α-acid and β-acid content for hops and hop pellets, the data is not shown here. For hops and hop pellets we could not find an extraction protocol that could be commonly used for both UV and HPLC analysis that would give statistically comparable results to the current approved ASBC methods.

Conclusion:
A methanol extraction protocol can be used in the extraction of CO_2 hop extracts for UV analysis, and will provide statistically equivalent results to the current extraction protocol in ASBC Hops-8. If one is analyzing CO_2 hop extracts by UV and HPLC then they could reduce preparation time and solvent waste by using a unified methanol extraction protocol for both analysis.

For the extraction of hops and hop pellets a unified sample extraction protocol did not produce statistically equivalent results. For the extraction of hops and hop pellets an analyst should use the extraction protocols given in the ASBC methods for UV and HPLC analysis. If one uses a unified extraction protocol for hops and hop pellets they will most likely achieve analytical results that will not be statistically comparable to the ASBC hop methods.

Acknowledgements:
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References:

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