Analysis of Hop Bittering Constituents

Subcommittee Members: M. H. Schwiesow, *Chairman*; J. Boersma, R. Burkhardt, M. Constant, H. L. Grant, R. W. Gross, E. Knudson, K. Markl, G. Nickerson, D. Ordemann, I. Rosendal (*EBC*), and L. Marinelli (*ex officio*).

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CONCLUSIONS

1. The ratios of the respective reference standard peak areas to the α- and β-acids peak areas generally showed lower coefficients of variation at 325 nm than at 254 nm.
2. The ratios of the α- to β-acids peak areas also showed lower coefficients of variation at 325 nm than at 254 nm.
3. In spite of several problems confronted by collaborators, an anion-exchange high performance liquid chromatography (HPLC) system appears to be a viable method to analyze for α-and β-acids in kettle hop extracts.

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RECOMMENDATIONS

1. The subcommittee should continue work using the anion-exchange HPLC system with the 325 nm variable-wavelength or the 313 nm fixed-wavelength ultraviolet (UV) detector.
2. The subcommittee should try to resolve the concerns noted by collaborators in this year's work.
3. The subcommittee should consider using an internal reference

TABLE I
Reference Standard Peak Area/Hop Acids Peak Area at 254 NM

	Samples							
	β-Acids				α-Acids			
Collaborator	A	B	C	D	A	B	C	D
1	45.69	42.13[a]	27.56	27.56	16.12	16.40	16.01	17.05
2	41.48	33.33	25.30	25.26	13.59	12.38	13.03	13.36
3	39.22	32.86	23.91	24.14	18.67	17.11	16.95	17.98
4	35.04	31.09	23.28	22.30	9.88	10.18	10.74	10.53
5	...	...	...	...	...	...	...	...
6	43.89	31.17	23.12	24.79	17.81	16.47	14.83	16.53
7	37.11	31.42	26.16	25.40	12.51	12.21	13.04	13.46
8	38.32	32.22	24.63	24.45	10.58	9.97	10.69	10.97
9	40.35	32.14	25.40	25.92	12.53	12.06	11.55	12.23
Mean[b]	39.34	32.03	24.92	24.98	13.96	13.35	13.36	14.01
Grand mean[b]	35.69		24.95		13.65		13.68	

[a] Outlier according to Dixon's test, $P \leq 0.05$ (1).
[b] Means do not include values for sample pairs containing outliers.

TABLE II
Reference Standard Peak Area/Hop-Acids Peak Area at 325 NM

	Samples							
	β-Acids				α-Acids			
Collaborator	A	B	C	D	A	B	C	D
2	3.55	2.83	2.14	2.15	1.64	1.50	1.59	1.65
4	4.15	3.53	2.63	2.57	1.74	1.69	1.78	1.76
6	3.20	2.65	1.97	1.95	2.00	1.89	1.92	2.00
7	3.44	2.79	2.12	2.15	1.64	1.53	1.62	1.70
8	3.69	3.00	2.25	2.28	1.53	1.43	1.51	1.56
Mean	3.61	2.96	2.22	2.22	1.71	1.61	1.68	1.73
Grand mean	3.28		2.22		1.66		1.71	

TABLE III
Statistical Summary of High Performance Liquid Chromatography Analyses of Hop Components and Their Relationship to a Reference Standard

Wavelength (nm)	Hop Acids	Sample Pair	No. of Labs	Grand Mean[a]	Laboratory Error			c.v.[d]	Calc. F[b]	Critical F[e]
					Within[b]	Between[b]	Combined[c]			
254	β	A-B	7	35.69	1.98	0.83	2.15	6.0	1.4	4.28
254	α	A-B	8	13.65	0.50	3.03	3.08	22.5	74.1	3.79
254	β	C-D	8	24.95	0.58	1.40	1.51	6.1	12.7	3.79
254	α	C-D	8	13.68	0.42	2.59	2.62	19.2	78.1	3.79
325	β	A-B	5	3.28	0.05	0.34	0.35	10.6	110.6	6.39
325	α	A-B	5	1.66	0.02	0.18	0.18	10.9	121.8	6.39
325	β	C-D	5	2.22	0.03	0.24	0.24	10.8	154.3	6.39
325	α	C-D	5	1.71	0.03	0.16	0.17	9.7	63.1	6.39

[a] Grand mean = GM = $(\bar{A} + \bar{B})/2$ or $(\bar{C} + \bar{D})/2$.
[b] Calculated per Youden and Steiner (4).
[c] Combined-laboratory error (S_c) calculated from within-laboratory error (S_r) and between-laboratory error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[d] Coefficient of variation of S_c = c.v. = 100 (S_c/GM).
[e] Critical F from tables of F distribution (2) at $P \leq 0.05$.

standard as an alternative to analyzing the reference standard separately.

For several years, this subcommittee has been concerned with the qualitative and quantitative analyses of hop acids in hops, hop pellets, kettle hop extracts, post-fermentation extracts, wort, and beer. The objective of the subcommittee was to consider the possible analytical techniques, explore and screen the efficiencies of the methods, and select a quick and reliable procedure by which the hop acids could be measured in the aforementioned list of media. A procedure that utilizes an anion-exchange HPLC system and very small amounts of hop acids was described by Gross and Schwiesow (3) and was the basis of this year's collaborative work.

EXPERIMENTAL

Two samples each of nonisomerized, solvent-extracted, kettle hop extract, and CO_2 hop extract were sent to collaborators along with reference standards. For those collaborators utilizing a fixed-wavelength UV detector at 254 nm, *p*-hydroxybenzoic acid was used as the reference standard. For the collaborators who had variable-wavelength UV detectors, 2,5-dihydroxybenzoic acid was used at 325 nm. Collaborators with the variable-wavelength UV detector were sent both standards and were requested to analyze the samples at both the above wavelengths.

TABLE IV
Ratio of α-Acids to β-Acids Peak Areas

	Samples							
	254 nm				325 nm			
Collaborator	A	B	C	D	A	B	C	D
1	2.83	2.57	1.72	1.62	...	...	...	...
2	3.05	2.69	1.94	1.89	2.16	1.88	1.34	1.30
3	2.10	1.92	1.41	1.35	...	...	...	...
4	3.55	3.05	2.17	2.10	2.39	2.08	1.48	1.45
5	2.84	2.86	1.89	1.86	2.03	1.82	1.27	1.31
6	2.46	1.89	1.56	1.50	1.60	1.40[a]	1.02	0.98[a]
7	2.97	2.58	2.01	1.89	2.10	1.82	1.31	1.26
8	3.62	3.23	2.30	2.23	2.42	2.10	1.49	1.46
9	3.22	2.67	2.20	2.12	...	...	...	...
Mean[b]	2.96	2.61	1.91	1.84	2.22	1.94	1.38	1.36
Grand mean[b]	2.78		1.87		2.08		1.37	

[a] Outlier according to Dixon's test, $P \leq 0.05$ (1).
[b] Means do not include values for sample pairs containing outliers.

Collaborators were requested to purchase a Vydac anion-exchange column and to condition the column before use. Hop extract samples were to be dissolved in methanol before injection on the isocratic HPLC system, which used a pH-adjusted methanol-water-acetate mobile phase. Weights, dilutions, retention times, and peak areas were requested, as were comments concerning difficulties or suggestions.

RESULTS AND DISCUSSION

The ratios of the reference standard peak areas to the hop acid peak areas for the 254-nm wavelength measurement are given in Table I. Similar ratios for the 325 nm wavelength are given in Table II. To compare the wavelengths and the chosen standard, all ratio values were subjected to Youden Unit Block statistical analysis. The statistical summary is given in Table III. Even though the coefficient of variation (c.v.) of the β-acids at 254 nm was lower than the 325 nm values, the α-acid c.v. at 254 nm was substantially higher than any 325 nm c.v. value. Because the subcommittee intends to measure α- and β-acids, a more precise methodology is desired.

To check whether the reference standards were acceptable, the ratio of α- to β-acids peak area was calculated for each collaborator (Table IV) and the ratios at both wavelengths were subjected to the Youden format. The statistical summary of the results is given in Table V. At the 325-nm wavelength, one collaborator was excluded as an outlier. The results of the other collaborators indicate substantially lower c.v. values at the 325-nm than at the 254-nm wavelength. For this reason, the 325-nm wavelength approach is desirable; however, some attempt to improve the reference standard should be made.

A large between-laboratory error was noted in both statistical surveys as compared to the within-laboratory errors. This implies that a more thorough examination of the procedure is needed. Even though several collaborators had essentially no problems with the procedure, others were confronted by numerous concerns, including: excessive back pressure from plugged columns, multiple hop-acid peaks, peak tailing, and poor hop-acid separation. In spite of these concerns, results from the collaborative study were encouraging.

LITERATURE CITED

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TABLE V
Statistical Summary of High Performance Liquid Chromatography Ratio of α-Acids to β-Acids Peak Areas

Wavelength (nm)	Sample Pair	No. of Labs	Grand Mean[a]	Laboratory Error			c.v.[d]	Calc. F[b]	Critical F[e]
				Within[b]	Between[b]	Combined[c]			
254	A-B	9	2.78	0.13	0.45	0.47	16.8	23.4	3.44
254	C-D	9	1.87	0.02	0.30	0.30	16.0	457.6	3.44
325	A-B	5	2.08	0.03	0.16	0.16	7.6	53.2	6.39
325	C-D	5	1.37	0.03	0.09	0.10	7.0	26.9	6.39

[a] Grand mean = GM = $(\bar{A} + \bar{B})/2$ or $(\bar{C} + \bar{D})/2$.
[b] Calculated per Youden and Steiner (4).
[c] Combined-laboratory error (S_c) calculated from within-laboratory error (S_r) and between-laboratory error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[d] Coefficient of variation of S_c = c.v. = 100 (S_c/GM).
[e] Critical F from tables of F distribution (2) at $P \leq 0.05$.