NOTE

High Performance Liquid Chromatographic Analysis of Hop Alpha- and Beta-Acids[1]

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ABSTRACT

A high performance liquid chromatographic method for the determination of α-acids (humulones) and β-acids (lupulones) in hops, hop pellets, and nonisomerized hop extracts is introduced. Rationale for the separation mode and conditions are given.

Key words: *α-Acids, β-Acids, High performance liquid chromatography, Hop analysis*

The bitter flavor found in beer is primarily due to iso-α-acids. These compounds are derived from the corresponding α-acids found in hops. The α-acids can be introduced into the beer by adding hops, hop pellets, or a nonisomerized hop extract to the wort during the kettle boil. An assay method is needed to determine the amount of α-acids in the hopping constituent that ensures a desired level of bitterness in the finished beer.

Several analytical techniques are currently used. Unfortunately, each has one or more serious drawbacks. The ultraviolet (UV) spectrophotometric method (1) determines the α-acids concentration in hops, hop pellets, or nonisomerized extract based on measurements of the absorbance of an alkaline methanolic solution at three wavelengths. Since many of the compounds in hops besides the α-acids absorb in the UV region, the method is susceptible to interferences. The conductiometric method (2) is not specific for α-acids, and the chemicals required for the analysis are toxic. The Dowex (3) and Sephadex (4) ion-exchange chromatographic methods represent systems in which components of the hops are separated from other UV-absorbing compounds before quantitation. However, these methods are time-consuming.

A rapid method employing anion-exchange high performance liquid chromatography (HPLC) was developed in our laboratory and used to analyze and compare α-acids in hops, hop pellets, and both organic solvent and carbon dioxide-extracted nonisomerized hop extracts. This method is expeditious and provides adequate separation to measure the α- and β-acids without interference from other UV-absorbing compounds present in hops.

EXPERIMENTAL

Reagents
(a) Methanol (HPLC grade)
(b) Water (HPLC grade)
(c) Sodium acetate (Reagent grade)
(d) 2,5-dihydroxybenzoic acid
(e) Toluene
(f) Acetic acid

Apparatus
(a) Isocratic HPLC with variable UV detector and integrator
(b) Anion-exchange HPLC column (Vydac™ 301-TP, 3.2 mm × 25 cm i.d.) (SEP/A/RA/TIONS Group, Vydac™, Hesperia, CA)

[1]From a presentation made at the 47th Annual Meeting, Miami, FL, May 1981.

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Chromatographic Conditions
Column: Vydac Anion Exchange 301-TP, 10μ particle size, 3.2 mm × 25 cm i.d.
Column temperature: 30° C
Solvent: 70/30 Methanol/0.015*M* aqueous sodium acetate at pH 5.2
Flow rate: 1.0 ml/min
Detector: UV at 325 nm

METHOD

For the analysis of α- and β-acids in hops or hop pellets, a toluene solution is prepared in the same manner as outlined in the ASBC spectrophotometric procedure (1). A clear aliquot is

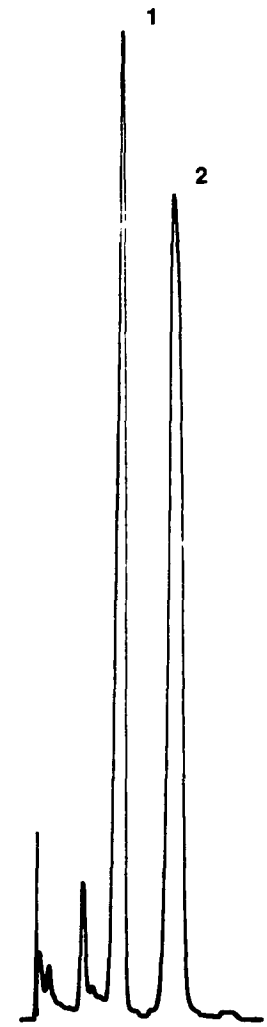

Fig. 1. Typical chromatogram obtained in analysis of a domestic hop. Peak 1 represents β-acids; peak 2 represents α-acids.

removed and accurately diluted with methanol to give a concentration of α-acids of approximately 50 mg/L. For nonisomerized hop extracts, the extract is diluted in methanol to give a concentration of α-acids of approximately 50 mg/L. The final dilution is centrifuged or filtered before injection to remove particles that could plug the column.

The mobile phase for the HPLC analysis is prepared by dissolving 2.07 g of sodium acetate trihydrate in 300 ml of HPLC-grade water. The pH is adjusted to 5.2, using 20% (v/v) acetic acid before adding 700 ml of HPLC-grade methanol. The solvent is filtered to degas before use.

The samples are analyzed by injecting 20 μl onto the column, using the previously described conditions. Response factors can be determined either by injection of a purified α- or β-acids standard or by comparisons of results to values obtained for a sample analyzed using ion-exchange chromatography (3,4).

If desired, a standard can be prepared by diluting 50 mg of 2,5-dihydroxybenzoic acid in 1 L of methanol. This can assure consistent performance of the HPLC unit or as a basis of comparison for results from different HPLC units.

RESULTS AND DISCUSSION

The anion-exchange mode of separation was chosen because a single peak elution of α-acids was desired. In reverse phase systems, separation is based on solubility. The solubility of the various α-acids depends on the composition of the side chain; therefore, humulone and cohumulone elute separately.

A typical chromatogram using the described chromatographic system is shown in Fig. 1. All of the β-acids are eluted in peak 1, followed by all of the α-acids in peak 2. The best quantifications of α- and β-acids were attained using the aforementioned conditions. The replicate analysis data in Table I shows good reproducibility, especially with the HPLC method. Somewhat higher values found by the spectrographic method may be caused by interferences.

Changes in the composition of the mobile phase can be used to change the elution profile. For example, additional water extends the retention time, and separation of the individual α- or β-acids is attained. This system can also be used to provide information on the reaction products of α-acids obtained in the brew kettle.

TABLE I
Replicate Analysis of Typical Domestic Hops by HPLC, and by Spectrophotometry[a]

	HPLC (%)		Spectrophotometry (%)	
Trial	α-Acids	β-Acids	α-Acids	β-Acids
1	6.17	4.76	6.48	4.70
2	6.10	4.78	6.60	4.78
3	6.05	4.73	6.11	5.05
4	6.10	4.76	6.45	4.88
5	6.15	4.78	6.18	5.03
6	6.04	4.71	6.38	4.78
Average	6.10	4.75	6.37	4.87
S.D.[b]	0.052	0.028	0.187	0.144
Percent c.v.[c]	0.85	0.59	2.93	2.95

[a] Method was HOPS-6, A(1).
[b] S.D. = standard deviation.
[c] c.v. = coefficient of variation.

LITERATURE CITED

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