BIO-RAD

Capillary Electrophoresis
Application Note 49

Analysis of Hop Bitter Acids by Micellar Electrokinetic Capillary Chromatography

Introduction

The common hop (*humulus lupulus*) is a plant used for centuries in the brewing of beer. Germany is the largest grower of hop, followed by the United States. Only the female plant, with its cone-like flower, is used in brewing. The tiny golden cones contain the characteristic hop resins and oils which give beers their distinctive character. Hop bitter acids are constituents of the hop resin fraction (~ 12 %) and impart most of the refreshing, clean bitterness to beers. The six major hop bitter acids are classified as α-acids (humulones: humulone, adhumulone, cohumulone) and β-acids (lupulones: lupulone, adlupulone, colupulone). The structures of these molecules are given in Figure 1. The normal- and adisomers differ only in the position of methylene groups, representing a challenging analytical problem. Chemical analysis of hop extracts is used for raw material characterization as well as for quality control purposes. In this Application Note, the analysis of hop bitter acids by micellar electrokinetic chromatography (MECC) is reported.

Results

Figure 2 shows the electropherogram of a mixture of the six major hop bitter acids. All peaks are well resolved with a run time of only 12 min. The β-acids (lupulones) have a higher molecular weight than the α-acids (humulones) and, consequently, have a lower mobility. Because the analytes move against the electro-osmotic flow, the β-acids elute earlier than the α-acids. The peaks eluting in the 5-7 min range are oxidized products. Temperature and buffer conditions (pH, buffer, and SDS concentration) are critical with respect to resolution and were carefully optimized. According to the provider of the sample, the peak marked with an arrow is indicative of the quality of the hop extract. Spectral information presented in the 3-dimensional plot (absorbance vs time vs wavelength) of Figure 3 is useful in the identification of unknown peaks.

Although it is possible to separate hop bitter acids with reversed phase HPLC, the separation takes about 45 min and involves the use of organic solvents as mobile phase modifiers. Capillary electrophoresis in the MECC mode is faster, more efficient, and requires no organic solvents. In addition, the MECC method is more economical for routine work.

Analysis Conditions

Instrument	BioFocus® 3000 system
Polarity	+ to -> negative
Capillary	50 µm x 50 cm, uncoated
Run buffer	100 mM sodium borate, 100 mM SDS, pH 8.3
Injection	pressure 10 psi * s
Run voltage	18 kV
Detection	214 nm
Cartridge temperature	30 °C
Autosampler temperature	10 °C
Sample	Commercial hop extract, 150 mg/ml methanol, diluted 1:100 in run buffer prior to injection

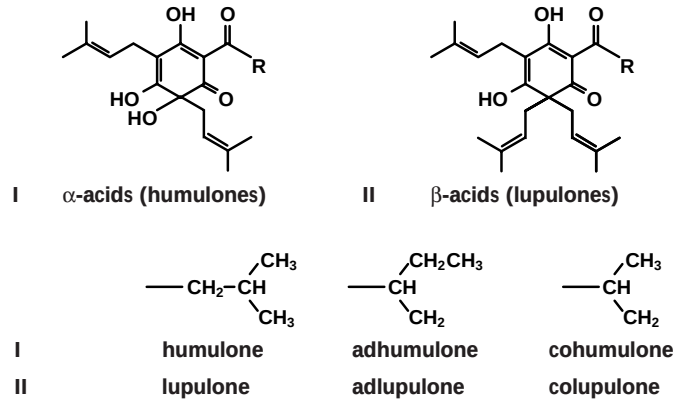

Fig. 1. Structures of hop bitter acids.

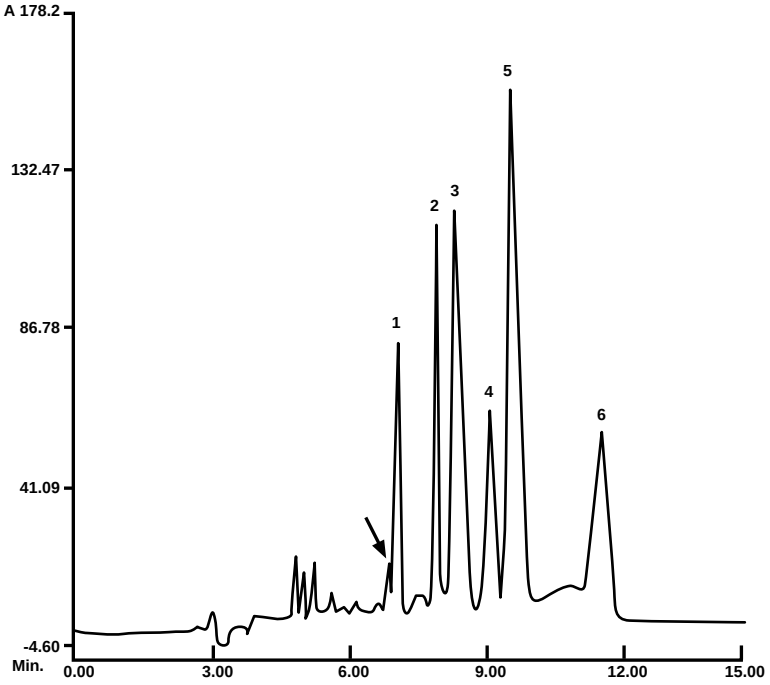

Fig. 2. Separation of six hop bitter acids by MECC. Peak identities: 1. colupulone; 2. lupulone; 3. adlupulone; 4. cohumulone; 5. adhumulone; 6. humulone.

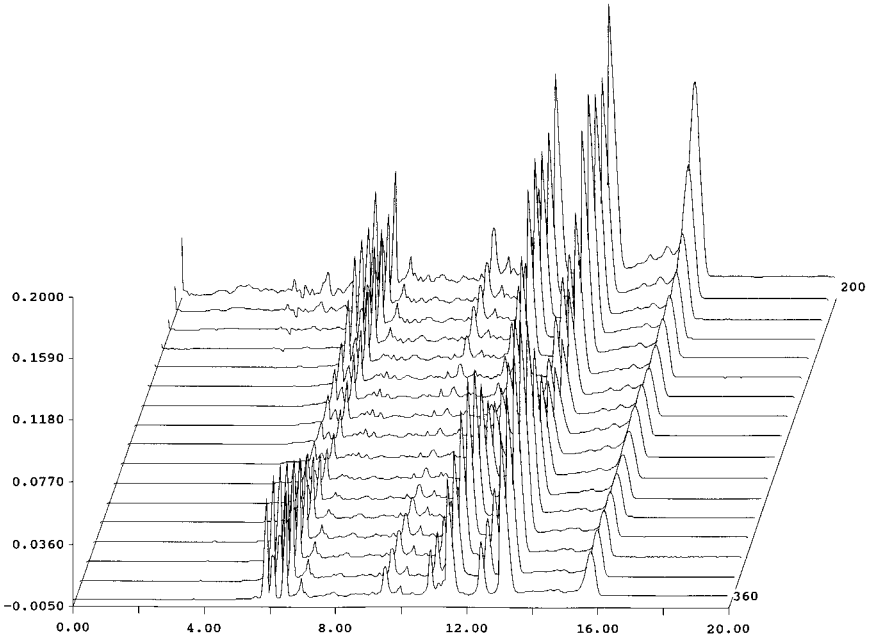

Fig. 3. 3-dimensional plot of a sample run at 20 °C.

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