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Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0054 - Hops for Bitter Acids by HPLC

Botanical Name: *Humulus lupulus*

Parts of Plant Used: Dried pistillate inflorescence/strobiles

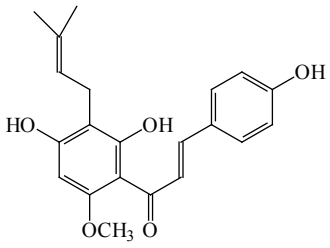
Uses: As a sedative to treat restlessness and insomnia.

Modes of Action:

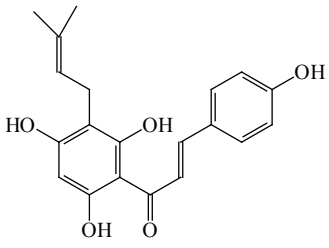
Hops, a well-known flavoring and preservative ingredient in the brewing of beer, also is bioactive. Clinical trials have proven the sedative effect of the hops extract. Although there were reports about the sedative-hypnotic activity of one component of hops oil (2-methyl-3-buten-2-ol, which is formed through the degradation of bitter acids, that is, α-and ß-acids), the components responsible for the activity of hops are still unknown.[1–3]

Chemical Markers:

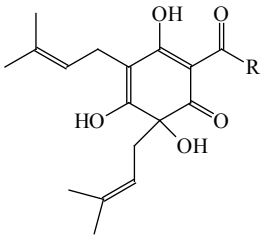
Hops contains numerous phytochemicals. The bitter acids, which are responsible for the characteristic bitterness of hops, are the main component. Their concentration usually is greater than 15% and can reach 30%. Two types of bitter acids are present in the plants: α-acids (humulone, cohumulone, adhumulone) and ß-acids (colupulone, lupulone, adluputone).[4] During beer brewing, the α-acids are converted to iso-α-acids, which impart to beer its typical bitter taste. Hops is known to contain flavonoids such as rutin and quercitrin, isoprenylated flavonoids such as 8-prenylnaringenin, isoxanthohumol, and xanthohumol.[5,6] Several flavonoids were identified in hops by MS and LC–MS and proanthocyanidins also were found in hops.[7–9] Hops also contains 0.3% to 1% oil, which is a complex mixture of hydrocarbons, esters, aldehydes, ketones, and alcohols.[10] The bitter acids (six major ones) are used as marker compounds for quality control of hops extract in the U.S. market.



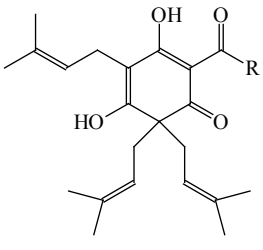
Xanthohumol



Dexmethylxanthohumol



Cohumulone: R = $CH(CH_3)_2$
Humulone: R = $CH_2CH(CH_3)_2$
Adlumulone: R = $CH(CH_3)CH_2CH_3$



Colupulone: R = $CH(CH_3)_2$
Lupulone: R = $CH_2CH(CH_3)_2$
Adlupulone: R = $CH(CH_3)CH_2CH_3$

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Methods of Analysis

Hops is an important flavoring agent for beers, and various methods have been used for the analysis of hops, especially the bitter acids. Due to the instability of the bitter acids of hops, GC is not a good method. HPLC and capillary electrophoresis (micellar electrokinetic chromatography and microemulsion electrokinetic chromatography) are suitable for bitter acids analysis.

Method 1:

The method of Hermans-Lokkerbol and Verpoorte was used.[11]

Sample Preparation:

Dissolve the sample in methanol and centrifuge before injection.

Chromatography:

Column: Phenomenex Hypersil 5 C18, 250 × 4.6 mm.
Mobile phase: 0.05 M Triethanolamine in water–methanol (35:65 vol/vol), pH adjusted to 6.86 with phosphoric acid.
Flow rate: 1.75 mL/minute
Injection volume: Not available
Detection wavelength: 325 nm for α-acids and 358 nm for ß-acids.

Validation Data:

LOD/LOQ: 25, 20, and 24 ng for humulone, cohumulone, and adhumulone, respectively, and 46, 33, and 41 ng for corresponding lupulones.

Method 2:

The method of De Keukeleire et al. was used.[12]

Sample Preparation:

Grind a hops sample (250 mg to 1 g), suspend in 10 mL of methanol of containing 0.01% formic acid, and stir for 1 hour.

Chromatography:

Column: Varian Omnisphere C18, 250 × 4.6 mm.
Mobile phase: Solvent A = water (0.025% formic acid), solvent B = methanol (0.025% formic acid).
Gradient:

Time (minutes)	%A	%B
0	55	45
3	55	45
32	5	95
37	5	95
45	55	45
47	55	45

Flow rate: 1 mL/minute
Injection volume: 20 µL
Detection wavelength: 314 nm for α- and ß-acids and 370 nm for xanthohumol and desmethylxanthohumol
Column temperature: 35°C

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Method 3:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

Sonicate about 200 mg of hops extract in 35 mL of methanol for 20 minutes, cool to room temperature, and fill to 50 mL.

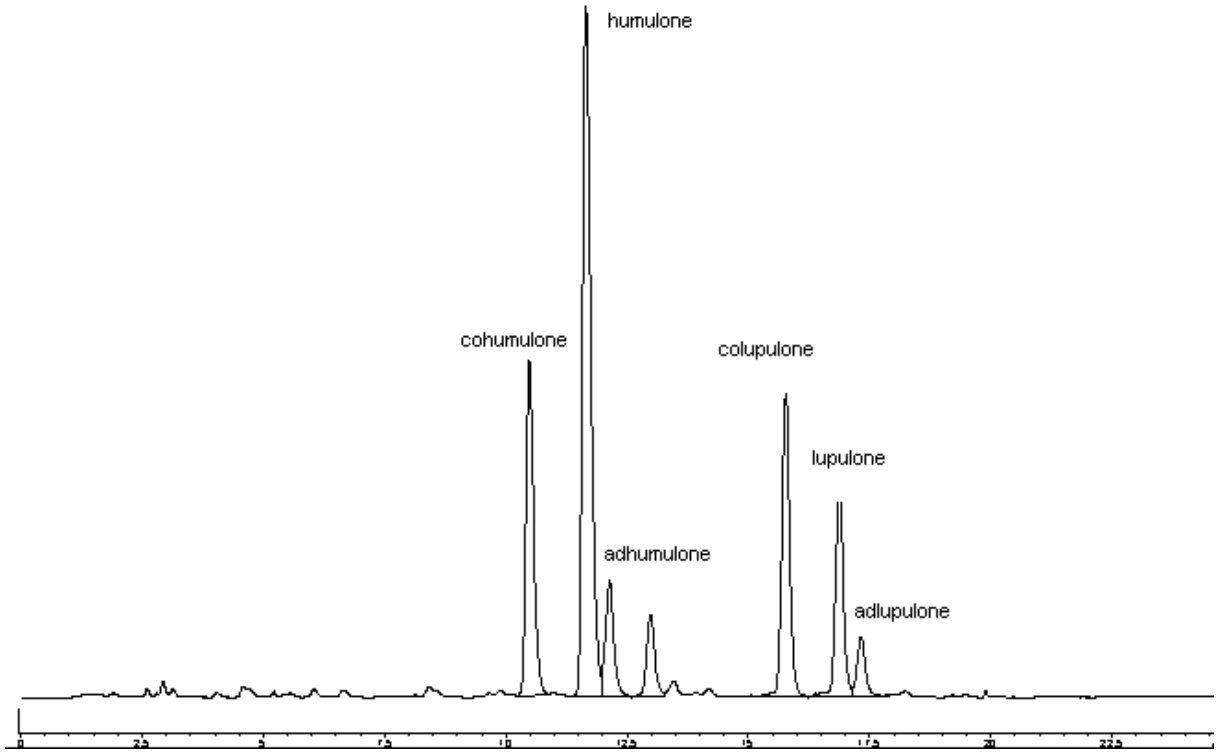
Chromatography:

Column: Phenomenex Phenylhexyl, 3 µm, 4.6 × 150 mm, with guard cartridge.
Mobile phase: Solvent A = water (0.1% formic acid) solvent B = acetonitrile (0.1% formic acid).
Gradient:

Time (minutes)	%A	%B
0	40	60
4	40	60
15	25	75
25	10	90

Flow rate: 1.0 mL/minute
Detection wavelength: 330 nm
Injection volume: 10 µL
Column temperature: Ambient

Representative HPLC Chromatogram Run by Method 3



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