

International Calibration Standards

for HPLC Analysis of Humulinones

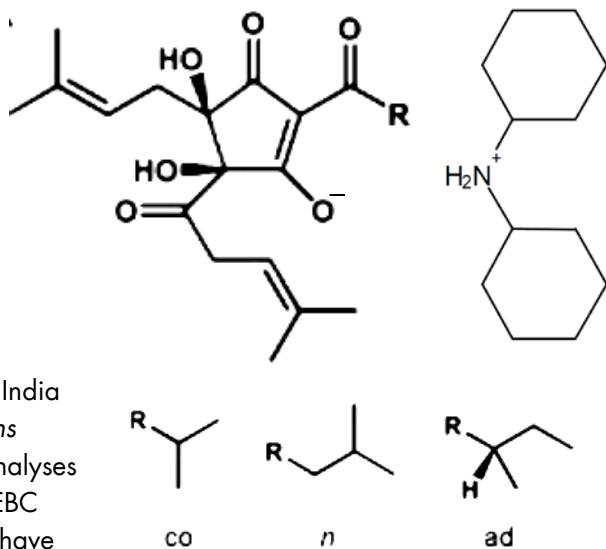
DCHA-Humulinones, ICS-Hum 1

ICS-Hum 1 is a purified preparation of the dicyclohexylamine salts of *trans*-humulinones.

Humulinones : 65.6 %(w/w)

The above concentration was determined by the International Hop Standards Committee (IHSC) and takes into account only the three major forms of the humulinones that are present: *trans*-cohumulinone, *trans*-humulinone and *trans*-adhumulinone.

Hops, hop pellets and beers (especially dry hopped India Pale Ales) will contain humulinones and only the *trans* forms of humulinones have been identified. HPLC analyses should be done using the recommended method of EBC 7.9 at a wavelength of 270 nm. Since humulinones have UV spectra very similar to that of the iso- α -acids, the extinction coefficients for all forms of the humulinones are believed to be all quite similar using the mobile phase of the recommended method.



When using ICS-Hum 1 for the calibration of HPLC, first determine the total area of the peaks corresponding to the above-mentioned three compounds on each of your calibration runs, then set the integrator by calculating and applying the same response factor to each one of the peaks.

If you are using the recommended method of EBC 7.9, expect the area of the *trans*-cohumulinone peak to be about 33.2% of the total peak area of all three HPLC peaks included in the calibration. (Caution: This may not be the case for methods that use other mobile phases or for measurement at different wavelengths).

The following two chromatograms illustrate (page 2) the three major peaks upon which the calibration must be based and (page 3) the minor peaks that are also present in the preparation. The spectra of all the peaks, as obtained from a photo-diode array (PDA) detector scanning at the peak maxima, are also shown. Some of these peaks may be *trans* forms of minor humulinones, including *trans*-posthumulinone.

Storage: -20 °C, gas-tight under CO₂ or N₂

Country of origin/produced: Germany

Rev. 01/Nov 2018

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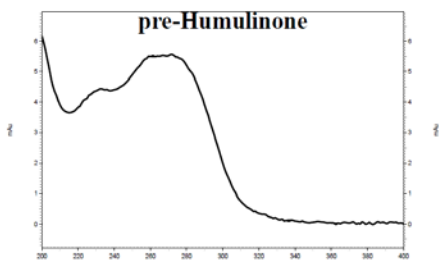
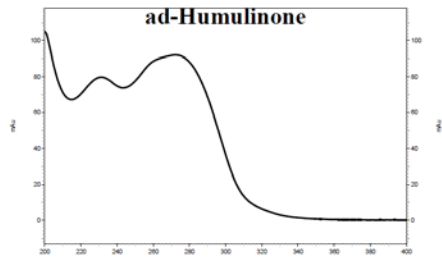
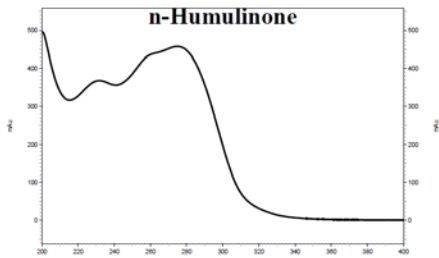
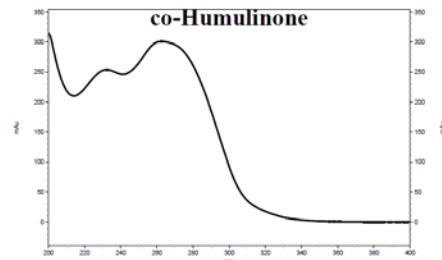
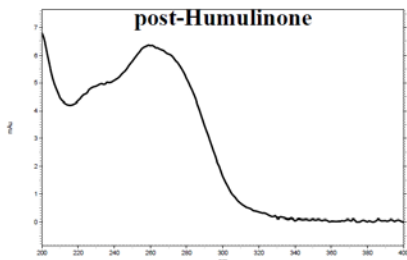
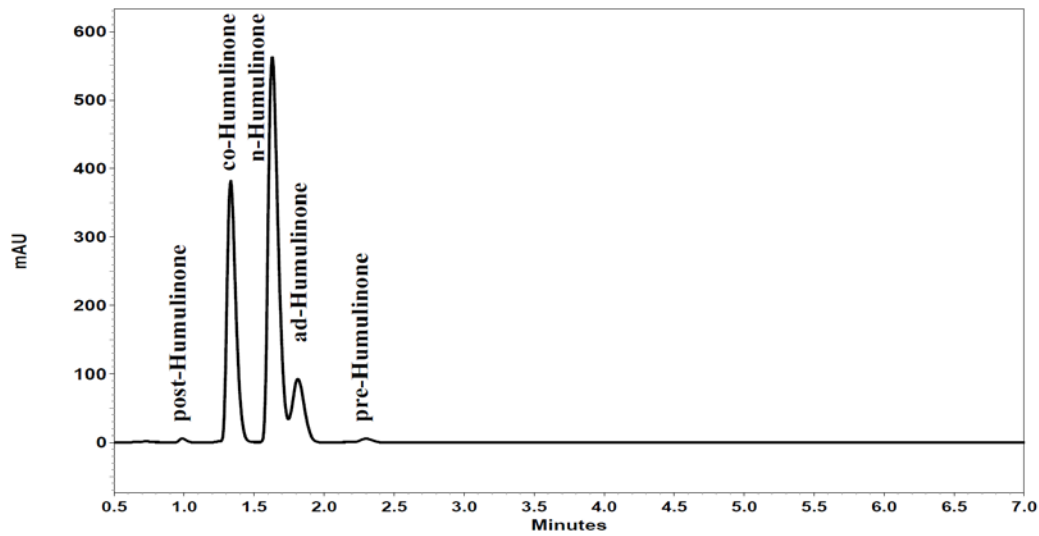
DCHA- Humulinones ICS-Hum1

International Hop Standards Committee

Data acquired by: Robert Smith, 8/21/18

Column: Restek Raptor ARC-18 2.7um 50 x 4.6mm, 1.3mL/min, 40C

Mobile Phase: 725mL MeOH / 265mL H₂O / 20 mL 85% H₃PO₄ / 0.75mL 1 N NaEDTA



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