



A User's Guide

to the new....

International Calibration Standards

for HPLC Analysis of Isomerized & Reduced Isomerized α -Acids

DCHA-Hexa, ICS-H1

ICS-H1 is a purified preparation of the dicyclohexylamine salts of *cis*-hexahydroiso- α -acids.

When used according to the accompanying instructions for storage, handling and use, especially including chromatography by the recommended, isocratic variation of Method EBC 7.8, this standard is deemed to have the following composition:

Total Hexahydroiso- α -acids: 65.7% (w/w)

The above figure was determined by the *International Subcommittee for Isomerized Hop α -Acids Standards* and takes into account **only** the major forms of hexahydroiso- α -acids that are present: two *cis*-hexahydroisocohumulones, two *cis*-hexahydroisohumulones and two *cis*-hexahydroisoadhumulones.

Samples of hop products containing hexahydroiso- α -acids, and hence the worts and beers made from them, will contain almost entirely *cis*-hexahydroiso- α -acids. Because these compounds are thought likely to all have quite similar extinction coefficients at 270nm in the mobile phase of the recommended method, ICS-H1 is considered suitable as a standard for all unknown samples.

When using ICS-H1 for the calibration of HPLC analysis, first determine the total area of the peaks corresponding to the above-mentioned six compounds on each of your calibration runs, then set the integrator by calculating and applying the **same** response factor to each one of these peaks.

If you are using the recommended method, expect the combined areas of the *cis*-hexahydroisocohumulones peaks to be about 53% of the total peak area of all of the compounds 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 (of a single analysis) illustrate (1) the (usually) **five** major peaks upon which the calibration must be based and (2) the minor peaks that are also present in the preparation. (**N.B.** For each pair of isomeric forms it is not known which of the two structures shown above corresponds to which of the peaks marked 1 and 2 on the first chromatogram). 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 *cis* forms of minor hexahydroiso- α -acids, some may be the corresponding *trans* forms of the major hexahydroiso- α -acids.

