



A User's Guide



to the new....

International Calibration Standards

for HPLC Analysis of Isomerized & Reduced Isomerized α -Acids

DCHA-Iso, ICS-I3

ICS-I3 is a purified preparation of the dicyclohexylamine salts of *trans*-iso- α -acids.

When used according to the accompanying instructions for storage, handling and use, especially including chromatography by the recommended Method EBC 7.9, this standard is deemed to have the following composition:

Total Iso- α -acids: 62.3% (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 the iso- α -acids that are present: *trans*-isocohumulone, *trans*-isohumulone and *trans*-isoadhumulone.

Conventionally produced worts and beers will contain both *trans* and *cis* forms of iso- α -acids, as too will samples of non-reduced, isomerized hop products and the worts or beers made from them. However, at 270nm the extinction coefficients for all forms of the iso- α -acids are believed to be all quite similar in the mobile phase of the recommended method, and hence ICS-I3 is considered suitable as a standard for all mixtures of isomers.

When using ICS-I3 for the calibration of HPLC analysis, 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, expect the area of the *trans*-isocohumulone peak to be about 33.6% 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 three major peaks upon which the calibration must be based and (2) 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 iso- α -acids, including *trans*-isoposthumulone, some may be *cis* forms of major iso- α -acids.

