



A User's Guide

to the new....

International Calibration Standards

for HPLC Analysis of Isomerized & Reduced Isomerized α -Acids

DCHA-Rho, ICS-R2

ICS-R2 is a purified preparation of the dicyclohexylamine salts of *cis*- ρ -iso- 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 7.9, this standard is deemed to have the following composition:

Total ρ -Iso- α -acids: 65.3% (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 ρ -iso- α -acids that are present: two *cis*- ρ -isocohumulones, two *cis*- ρ -isohumulones and two *cis*- ρ -isoadhumulones.

Hop products containing ρ -iso- α -acids, and hence the worts and beers made from them, have mostly *cis*- ρ -iso- α -acids but may also include a significant proportion of corresponding *trans*- isomers. Because all these compounds are thought likely to have quite similar extinction coefficients at 270nm in the mobile phase of the recommended method, ICS-R2 is considered suitable as a standard for all unknown samples.

When using ICS-R2 to calibrate HPLC analysis, first determine the total peak area corresponding to the above-mentioned six compounds on each calibration run, then set the integrator by calculating and applying the **same** response factor to each one of these peaks. Analysis by EBC 7.9 of commercial *Rho* products and beers containing them will often show a significant peak following the last of the major peaks in the standard. This peak is now known to be comprised primarily of *trans*-isohumulone and should be included in the calculation of total *Rho* content. Charts showing typical commercial products are included with the standard for reference purposes.

If you are using the recommended method, expect the combined area of the *cis*- ρ -isocohumulones peaks to be about 14.5% 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 appended 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 the most significant peaks, as obtained from a photo-diode array (PDA) detector scanning at the peak maxima, are also shown. Some of the minor peaks may be *cis* forms of minor ρ -iso- α -acids, some may be the corresponding *trans* forms of the major ρ -iso- α -acids.

