



## A User's Guide



*to the new....*

### *International Calibration Standards*

for HPLC Analysis of Isomerized & Reduced Isomerized  $\alpha$ -Acids

## Tetra, ICS-T2

ICS-T2 is a purified preparation of tetrahydroiso- $\alpha$ -acids containing both *cis* and *trans* isomers.

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 Tetrahydroiso- $\alpha$ -acids: 99.4% (w/w)

The above figure was determined by the *International Subcommittee for Isomerized Hop  $\alpha$ -Acids Standards* and takes into account **only** the major forms of the tetrahydroiso- $\alpha$ -acids that are present: two isomers (*cis* & *trans*) each of the tetrahydroisocohumulones, tetrahydroisohumulones and tetrahydroisoadhumulones.

Samples of hop products containing tetrahydroiso- $\alpha$ -acids, and the worts and beers made from them, will naturally contain a different balance of the various homologs and isomers. However, since these latter are believed to all have quite similar extinction coefficients at 270nm in the mobile phase of the recommended method, ICS-T2 is considered suitable as a standard for all unknown samples.

When using ICS-T2 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) area of the tetrahydroisocohumulones peak(s) to be about 39% 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) **three** major peaks upon which the calibration must be based and (2) the minor peaks that are also present in the preparation. (N.B. It is sometimes found that in the recommended method there is a partial split of the tetrahydroisocohumulones peak, in which case the first peak of the pair is believed to be the *trans* isomer). 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 minor tetrahydroiso- $\alpha$ -acids.

