International Calibration Standards for HPLC Analysis of Isomerized & Reduced Isomerized α-Acids

DCHA-Iso, ICS-I3 (Iso-α-acids standard)

DCHA-Rho, ICS-R2 (*Rho*-iso-α-acids standard)

Tetra, ICS-T2 (Tetrahydroiso-α-acids standard)

DCHA-Hexa, ICS-H1 (Hexahydroiso-α-acids standard)

History

In 1998, the ASBC, EBC, IoB (now IGB) and BCOJ began working together toward the creation and international adoption of a single set of HPLC standards for use in the quantitative determination of isomerized and reduced-isomerized α-acids in hop products and in beers. The resultant **International Calibration Standards (ICS)** were produced, analyzed, and verified for the benefit of the brewing industry by the International Sub-committee for Isomerized Hop α-Acids Standards and first released in 2001, since which time three of the original standards have now been superseded by new preparations.

Descriptions

- The four current standards are crystalline preparations containing *trans*-iso-α-acids (ICS-I3); *cis-p*-iso-α-acids (ICS-R2); *cis- & trans-* tetrahydroiso-α-acids (ICS-T2) and *cis*-hexahydroiso-α-acids (ICS-H1). Used as instructed, they are considered suitable for totaled, quantitative analysis of all normally encountered mixtures of *cis* & *trans* isomers and major homologs (co, n & ad forms).

- Excepting in the case of ICS-T2, these standards are presented in the form of their dicyclohexylamine (DCHA) salts. Their purities were initially measured using a variety of HPLC procedures, agreement between these different methods being considered acceptable.

- The standards were later re-analyzed using an isocratic version of EBC Method 7.8 (now issued as EBC Method 7.9), following which their total content of the major isomerized or reduced isomerized α-acids were determined as being:

DCHA-Iso, ICS-I3:	**62.3%**	(of which *trans*-isocohumulone = ~ 33.6% by relative peak area)
DCHA-Rho, ICS-R2:	**65.3%**	(of which *cis-p*-isocohumulones = ~ 14.5% by relative peak area)
Tetra, ICS-T2:	**99.4%**	(of which *cis- & trans-* tetrahydroisocohumulones = ~ 39% by relative peak area)
DCHA-Hexa, ICS-H1:	**65.7%**	(of which *cis*-hexahydroisocohumulones = ~ 53% by relative peak area)

- Instructions for use are supplied. For peak identification purposes, these include typical chromatograms of the standards themselves, as obtained by the recommended HPLC method,

- Comparative results from an 2-year stability test of standards held at freezer and room temperature indicated excellent stability under either temperature, although freezer storage is strongly recommended. *(The composition of these standards, as held under recommended storage conditions, will be monitored to ensure that their purity is maintained).*

Limitations

*Most HPLC methods will not reliably separate (if present) all cis- and trans- isomers of the three, major homologs (co, n & ad forms) of a given type of isomerized α-acid. These standards are therefore recommended primarily for the determination of the **total** isomerized α-acids of the stated type in an unknown sample - though it may often be possible to concurrently determine the approximate cohumulone homologs content.*

Furthermore, in some HPLC elution solvents it may be expected that the extinction coefficients of the different isomers and homologs are not at all similar, leading to substantial errors in quantification of an unknown sample. Especially, this may be the case if the detector is set to an inappropriate wavelength.

*Where different types of iso-α-acids are present together in an unknown sample (e.g. iso-α-acids plus p-*iso-α-acids*), then these may not be fully resolved by some HPLC methods, leading to possible errors of identification and hence of quantitative evaluation.*

ooo OOO ooo