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# New International Calibration Standard (ICE-3) for HPLC Analysis of $\alpha$ - and $\beta$ -acids

Submitted on behalf of the International Hop Standards Committee

**The International Hop Standards Committee (IHSC), announces the release of a new calibration standard, ICE-3, for the HPLC analysis of  $\alpha$ - and  $\beta$ -acids. This standard replaces ICE-2, which is now withdrawn.**

Descriptors: Iso- $\alpha$ -acids, *Rho*-iso- $\alpha$ -acids, Tetrahydroiso- $\alpha$ -acids, Hexahydroiso- $\alpha$ -acids, calibration standard, HPLC analysis, methodology

## Background

In 1996 the EBC and the ASBC released the first “International Calibration Extract” (ICE-1) for HPLC analysis of  $\alpha$ - and  $\beta$ -acids in hops and hop products. A joint EBC/ASBC Hop Standard Subcommittee was established to monitor the stability of ICE-1. The results of a check carried out in spring 1997 convinced the Subcommittee to plan the release of a new hop standard. To guarantee the best possible stability of the successor the Working Group of Hop Analysis (AHA) tested in extensive storage trials at  $-20^{\circ}\text{C}$  and  $+40^{\circ}\text{C}$  several extracts of varying composition each one filled in aluminum, tin, plastic or glass vials. On the basis of the results received it was decided to choose as best suited for ICE-2 the de-oiled supercritical  $\text{CO}_2$ -extract “Hallertau Perle” filled in glass vials. This standard was then released by September 1<sup>st</sup>, 1998. Since that time, the stability of ICE-2 has been checked every 6 months by the Working Group of Hop Analysis (AHA) and this standard proved to be stable for more than 10 years until today.

In 2001, the ASBC, EBC, *IoB* (now *IBD*), and *BCOJ* approved the release of a set of HPLC standards for use in the quantitative determination of isomerized and reduced-isomerized  $\alpha$ -acids in hop products and in beer:

- DCHA-Iso, ICS-I (Iso- $\alpha$ -acids standard);
- DCHA-Rho, ICS-R (*Rho*-iso- $\alpha$ -acids standard);
- Tetra, ICS-T (Tetrahydroiso- $\alpha$ -acids standard);
- DCHA-Hexa, ICS-H (Hexahydroiso- $\alpha$ -acids standard).

For the production, release and maintenance of this set of standards the International Subcommittee for Isomerized Hop  $\alpha$ -Acids Standards was founded working very successfully since that time. It is consisting of experts representing ASBC, EBC, IBD and BCOJ.

In June 2009, the ASBC and EBC asked this international subcommittee to also take on the responsibility for other standards used for HPLC analysis of hops including the standard for  $\alpha$ - and

$\beta$ -acids. Therefore the name was changed from *International Subcommittee for Isomerized Hop  $\alpha$ -Acids Standards* to *International Hop Standards Committee (EBC/ASBC/BCOJ/IBD)*. Currently this committee is jointly chaired by Bob Foster (Miller-Coors, USA) and Martin Biendl (Hopsteiner, Germany) both acting as co-chairman representing the Americas and the rest of the world respectively.

## Release of ICE-3

Stocks of ICE-2 are almost exhausted. In anticipation of this, in 2006 the Working Group of Hop Analysis (AHA) initiated the preparation of a replacement standard. Similar efforts were undertaken as in case of the selection of ICE-2. In the end again a de-oiled supercritical  $\text{CO}_2$ -extract “Hallertau Perle” was selected as the most suitable replacement material. The new standard was supplied by NATECO2 GmbH (Germany), under the guidance of AHA member Roland Schmidt. Packaging was also carried out in the same company. The committee records its considerable appreciation to Roland Schmidt and his colleagues for all their skilled input to the successful manufacture of this new standard.

Following preparation, in November 2009 members of the International Hop Standards Committee (IHSC) carried out a collaborative trial in order to validate the new standard and assign values to the concentration of the  $\alpha$ - and  $\beta$ -acids. This work included a collaborative HPLC study in which the prospective new standard was crosschecked against the existing standard.

***This new standard, ICE-3, becomes the recommended standard and should be used for commercial transactions as well as for quality control purposes.***

## Use of the New Iso Standard

As would be expected, the composition of ICE-3 is not identical to the standard it replaces, but it can be used in exactly the same way. No significant differences were observed when quantifying

$\alpha$ -acids using ICE-3 vs. ICE-2. Such differences can be expected to be within the normal range of experimental error.

But users will find that the ICE-3 results for  $\beta$ -acids will differ significantly from ICE-2. The magnitude of this difference can be expected to be a relative drop of around 5 % (i.e. the new standard gives a lower result).

However, the committee agreed that the use of ICE-3 will lead to a truer result than that obtained using ICE-2 as many efforts have now been undertaken to find a suitable primary standard for  $\beta$ -acids. For that purpose the dicyclohexylamine ("DCHA") salt of  $\beta$ -acids was prepared and supplied by IHSC member Bob Smith (Hopsteiner, USA). The purity of DCHA- $\beta$ -acids could be ascertained by elemental analysis. For  $\alpha$ -acids both "pure humulone" (prepared by AHA member Klaus Kammhuber, Huell, Germany) as well as o-phenylenediamine ("OPDA") salts of  $\alpha$ -acids (also prepared by Bob Smith, Hopsteiner, USA) served as primary standards. The results of the international collaborative trial obtained with these different types of primary standards were then compared to the results obtained with ICE-2 as external calibration standard. As in case of the  $\alpha$ -acids there were no significant differences but almost identical results the International Hop Standards Committee (IHSC) agreed to assign the  $\alpha$ -acids values of ICE-3 based on external calibration with ICE-2 whereas in case of  $\beta$ -acids the values were based on external calibration with dicyclohexylamine ("DCHA") salt of  $\beta$ -acids.

The following composition of ICE-3 has been determined accordingly and agreed on by the International Hop Standards Committee (IHSC):

Total  $\alpha$ -acids: 44.64 %

Cohumulone: 13.88 %

N+adhumulone: 30.76 %

Total  $\beta$ -acids: 24.28 %

Colupulone: 13.44 %

N+adlupulone: 10.84 %

### How to Purchase

Stocks of ICE-3 are being divided between ASBC (in USA) and EBC (distribution through *Labor Veritas* in Switzerland), from which sources analysts can purchase the new standard in the usual vials.\*

ICE-2 will now be available only while stocks last. ICE-3 is valid from September 1<sup>st</sup> 2010.

\* Purchasers in the USA, Canada, Central and South America should contact ASBC headquarters (email: [asbc@scisoc.org](mailto:asbc@scisoc.org)), while those in Europe and Africa should direct enquiries to Labor Veritas, Zürich, Switzerland (email: [admin@laborveritas.ch](mailto:admin@laborveritas.ch)). Persons ordering from other parts of the world may make their approach to either party.