





# International Calibration Extract 4 (ICE-4)

for HPLC Analysis of  $\alpha$ - and  $\beta$ -acids

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## **Description**

The ICE-4 is a hop extract containing a specified concentration of  $\alpha$ - and  $\beta$ -acids. It replaces the ICE-3 as the international calibration extract hop standard of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC).

### **Calibration**

At the meeting of the EBC Hops Subcommittee in June 2018, all data have been reviewed and discussed. The results look very positive and the statistical data are impressive (CVSR 1-3 % for all parameters). The differences between the calibration of ICE-4 using ICE-3 as primary calibration standard or the DCHA complexes of  $\alpha$ - and  $\beta$ -acids turn out to be totally insignificant.

Considering these results, the conclusion of the Executive Committee of the International Hop Standard Committee (IHSC) is to take the results from primary calibration with ICE-3 as basis for assessment of the ICE-4 composition (average values of totally 13 results both for  $\alpha$ - and  $\beta$ -acids): This composition is fully verified by calibration of ICE-4 using the two DCHA complexes (note: M. Biendl).

As would be expected, the composition of ICE-4 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-4 vs. ICE-3. Such differences can be expected to be within the normal range of experimental error.

The following composition of ICE-4 has been determined accordingly and agreed on by the *International Hop Standards Committee (IHSC)*:

International Calibration Extract 4 (ICE-4)			
Cohumulone	10.98 %	Colupulone	13.02 %
N+adhumulone	31.60 %	N+adlupulone	13.52 %
Total α-acids	42.58 %	Total β-acids	26.54 %

ICE-4 replaces ICE-3 and is valid from August 1st, 2018.

Country of origin/produced: Germany

Refined natural product. Intended use is for analytical purposes.

#### Storage

Store the calibration extract, flushed with CO<sub>2</sub> or N<sub>2</sub> at -20 °C.

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## Handling

For use, heat up the frozen extract to about 20 °C and homogenize the entire content of the jar well by stirring it vigorously with a spatula. Prepare a calibration extract solution according to EBC method 7.7 or ASBC Hops-14. To avoid frequent heating of the whole extract, the contents of the jar may be subdivided into smaller, single-use portions in the following manner: Heat up the frozen extract to about 20 °C and homogenize the entire content of the jar well by stirring it vigorously with a spatula. Distribute the whole calibration extract from the jar into individual glass vials as follows: weigh approximately 0.5 g of extract into individual glass vials and record weights to the nearest

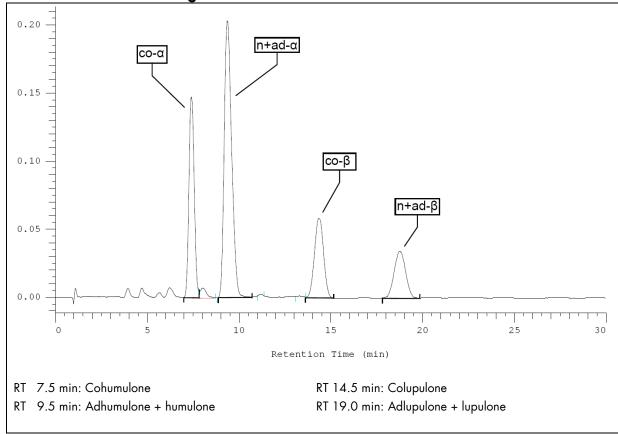
0.1 mg. Flush the vials with CO $_2$  or N $_2$ , close gas-tight and store in a freezer at -20 °C.

### Use of the ICE-4

ICE-4 may be used as a reference or control for the following methods of analysis:

EBC: Methods 7.7 and 7.8. ASBC Methods of Analysis: Hops-14 and Hops-16.

Reference HPLC-Chromatogram of ICE-4



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