International Calibration Extract 2 (ICE-2)

Description
ICE-2 is a hop extract containing a specified concentration of α- and β-acids. It replaces ICE-1 as the international calibration extract hop standard of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC). It was produced by extraction of Hallertau Perle hops with supercritical CO₂ and deoiling of the extract for improved stability. In addition to α- and β-acids, the standard contains uncharacterized soft resins, and small amounts of waxes and residual essential oils.

Calibration
In 1994, the EBC and the ASBC formed a joined subcommittee (EBC/ASBC Joint Hop Standard Subcommittee) to work toward the adoption of a single, consistent, international calibration extract hop standard for HPLC analysis of α- and β-acids in hops and hop products. The Subcommittee was established through cooperation between the EBC Analysis Committee and the ASBC Technical Committee. The EBC and ASBC released the first "International Calibration Extract" (ICE-1) for HPLC analysis of α- and β-acids in hops and hop products on July 1, 1996. As agreed at its introduction, the Joint Hop Standard Subcommittee monitored the stability of ICE-1. The results of a stability check carried out in the spring of 1997 convinced the Subcommittee to plan the release of a new international calibration extract hop standard. The Working Group for Hop Analysis (AHA) tested in extensive storage trials at -20 and +40 °C several extracts of varying composition, each one filled in metal cans, plastic and glass vials. On the basis of the results obtained, it was decided to choose deoiled supercritical CO₂ extract from Hallertau Perle, packaged in glass vials, for ICE-2. The α- and β-acids content of this extract were determined by collaborative analysis with participation by laboratories from EBC, ASBC and AHA, using as calibration standards pure a-acids ("pure humulone") for α-acids and ICE-1 for β-acids.

ICE-2 has the following composition:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Cohumulone</td>
<td>14.45%</td>
</tr>
<tr>
<td>Adhumulone + humulone</td>
<td>34.94%</td>
</tr>
<tr>
<td>Total α-acids</td>
<td>49.39%</td>
</tr>
<tr>
<td>Colupulone</td>
<td>12.92%</td>
</tr>
<tr>
<td>Adlupulone + lupulone</td>
<td>12.02%</td>
</tr>
<tr>
<td>Total β-acids</td>
<td>24.94%</td>
</tr>
</tbody>
</table>

ICE-2 replaces ICE-1 and has been valid since September 1, 1998.

Uses
ICE-2 may be used as a reference or control for the following methods of analysis:
Analytica-EBC: methods 7.7 and 7.8.
ASBC Methods of Analysis: hops-6,A; hops-6,B; hops-8,13 and hops-1 4.

Storage und Handling
Store the calibration extract, flushed with CO₂ or N₂ at -20 °C.
For use, heat up the frozen extract sample 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 Analytica-EBC method 7.7 or ASBC Hops-1 4. 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 0.6 g of extract into individual glass vials and record weights to the nearest 0.1 mg. Flush the vials with CO₂ or N₂, close gas-tight and store in a freezer at -20 °C.

11.09/Ld

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Reference HPLC-Chromatogram of ICE-2

RT  8.5 min: Cohumulone
RT 11.0 min: Adhumulone + humulone
RT 17.0 min: Colupulone
RT 22.3 min: Adlupulone + lupulone