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#7697EBC-2/09

Product	Price	Quantity	CHF Total
DCHA-Iso, ICS-I4	CHF 125		
DCHA-Rho, ICS-R3	CHF 125		
Tetra, ICS-T3	CHF 125		
DCHA-Hexa, ICS-H2	CHF 125		
ICE-3	CHF 250		
<b>GRAND TOTAL</b>			

No credit cards or checks accepted.  
Incoterms: DAP; Delivery on prepayment

### Payment to Labor Veritas AG:

Zürcher Kantonalbank, CH-8010 Zurich  
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# International Calibration Standards for HPLC Analysis of Isomerized $\alpha$ -Acids



Current from June 2017

## New International Calibration Standards for HPLC Analysis of Isomerized $\alpha$ -Acids

In 1998, the ASBC, EBC, IoB (now IBD), 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  $\alpha$ -acids in hop products and in beer. The resultant International Calibration Standards (ICS) were produced, analyzed, and verified for the benefit of the brewing industry by the International Subcommittee for Isomerized Hop  $\alpha$ -Acids Standards and first released in 2001.

The purity of each standard was determined using various HPLC procedures, elemental analysis, and other methods. The standards were later reanalyzed, using an isocratic version of EBC Method 7.8, following which the total concentrations of the major isomerized  $\alpha$ -acids were determined. Samples of the standards showed excellent stability even after two years at room temperature, although freezer storage is strongly recommended. New DCHA-Iso, DCHA-Rho, and Tetra standards (ICS-I4, ICS-R3 and ICS-T3) are now prepared, replacing the originals that are now available only while stocks last.

### General

- These standards are deemed to have the reported composition only 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 (now issued as EBC 7.9).
- Compared to these standards, samples of hop products, and the worts and beers made from them, may contain substantially different proportions of the constituent isomerized  $\alpha$ -acids. However, these latter compounds are believed to all have quite similar extinction coefficients at 270 nm in the mobile phase of the recommended method, hence the standards are considered suitable for use in quantifying isomerized  $\alpha$ -acids in all unknown samples.
- Detailed information pertaining to each standard, plus full instruction for use, is available on request and is supplied with each purchase, including: two chromatograms of a single analysis that illustrate (1) the major peaks upon which the calibration must be based and (2) the minor peaks that are also present in the preparation.

The spectra obtained from a photo-diode array (PDA) detector scanning at the peak maxima of all these peaks are also included.

### Limitations

- Most HPLC methods will not reliably separate all *cis*- and *trans*-isomers of the three major homologs (co-, n- & ad-) of a given type of isomerized  $\alpha$ -acid. These standards are therefore recommended primarily for the determination of the total content of isomerized  $\alpha$ -acids of the stated type in an unknown sample, though it may often be possible to concurrently determine the cohumulone homologs content.
- 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 mixtures of different types of iso- $\alpha$ -acids are present, they may not be fully resolved by some HPLC methods, leading to errors of identification and quantitative evaluation.

### Purchasing

These Iso standards can be purchased, at the equivalent price, from:

1. Labor Veritas AG, Zürich, Engimattstrasse 11 Postfach 353 CH-8027 Zürich, Switzerland.  
  
Phone: +41 (44) 283 29 30  
Fax: +41 (44) 201 42 49  
E-mail: admin@laborveritas.ch  
Web site: www.laborveritas.ch
- 2.
3. American Society of Brewing Chemists (ASBC): 3340 Pilot Knob Road St. Paul, MN 55121, USA.  
  
Phone: +1.651.454.7250  
Fax: +1.651.454.0766  
E-mail: asbc@scisoc.org  
Web site: www.asbcnet.org

Orders from Europe and Africa should be directed to Labor Veritas, from North and South America to ASBC, from elsewhere to either seller. LV reserves the right to limit the number of vials of each standard purchased.

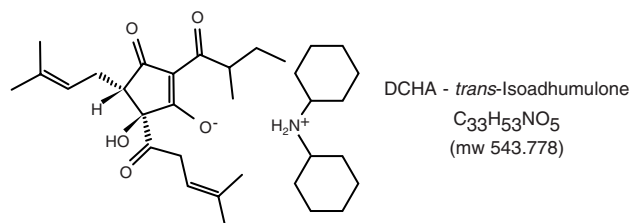
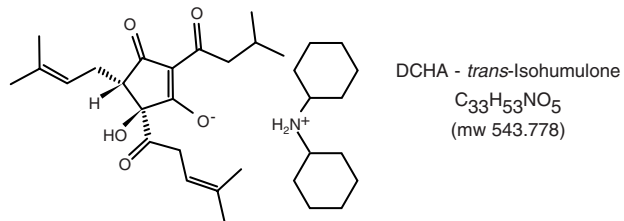
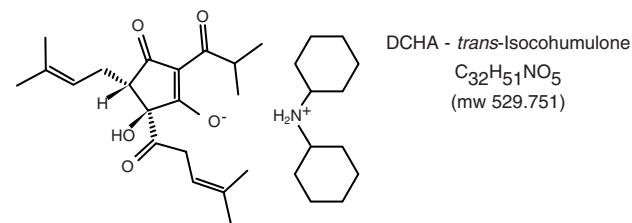
## DCHA-Iso, ICS-I4

ICS-I4 is a purified preparation of the dicyclohexylamine salts of *trans*-iso- $\alpha$ -acids. It is deemed to have a total iso- $\alpha$ -acids content of 65.2% (w/w), though this figure takes into account only the major forms of the iso- $\alpha$ -acids that are present: *trans*-isocohumulone, *trans*-isohumulone and *trans*-isoadhumulone. (N.B. Worts and beers brewed with hops, hop extracts, pellets and all commercial “Iso” products invariably also contain substantial proportions of the corresponding *cis*-iso- $\alpha$ -acids - see User’s Guide supplied with the standard).

If you are using the recommended method, expect the area of the *trans*-isocohumulone peak to be about 33.6% 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).

ICS-I4 replaces ICS-I3. The new standard has been cross-checked against the old and will give similar results.

### Structures of DCHA-Iso-alpha-acids



## DCHA-Rho, ICS-R3

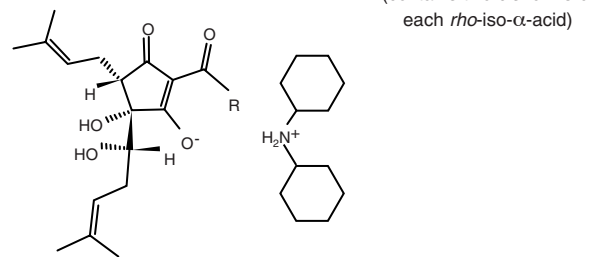
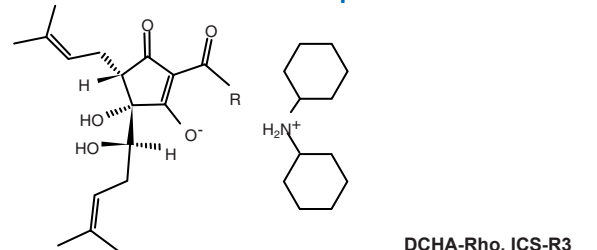
ICS-R3 is a purified preparation of the dicyclohexylamine salts of *cis*- $\rho$ -iso- $\alpha$ -acids. This standard is deemed to have a total  $\rho$ -iso- $\alpha$ -acids content of 65.0% (w/w), though this figure takes into account only the major forms of the  $\rho$ -iso- $\alpha$ -acids that are present: two *cis*- $\rho$ -isocohumulones, two *cis*- $\rho$ -isohumulones and two *cis*- $\rho$ -isoadhumulones. (N.B. Commercial “Rho” products typically contain a significant proportion of a *trans*- $\rho$ -isohumulone isomer - see User’s Guide supplied with the standard).

If you are using the recommended method, expect the combined area of the *cis*- $\rho$ -isocohumulones peaks\* to be about 14.5% 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).

ICS-R3 replaces ICS-R2. The new standard has been cross-checked against the old and has an essentially identical response factor per unit of total *cis*- $\rho$ -iso- $\alpha$ -acids.

\* *The supplied chromatogram shows two peaks, corresponding to the two cis forms of this particular iso- $\alpha$ -acid. However, it is not known which peak corresponds to which of the two forms shown below. (The same is also true for the  $\rho$ -isohumulones peaks).*

### Structures of DCHA-rho-Iso-alpha-acids



DCHA- $\rho$ -isohumulones & isoadhumulones  
C<sub>33</sub>H<sub>55</sub>NO<sub>5</sub>  
(mw 545.791)

## Tetra, ICS-T3

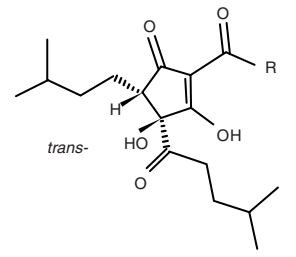
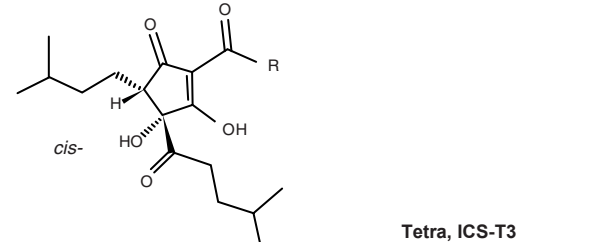
ICS-T3 is a purified preparation containing both *cis*- and *trans*-isomers of the tetrahydroisocohumulones, tetrahydroisohumulones and tetrahydroisoadhumulones. In respect of these six isomers it is deemed to have a total tetrahydroiso- $\alpha$ -acids content of 99.4% (w/w).

If you are using the recommended method, expect the (combined) area of the tetrahydroisocohumulones peak(s)\* to be about 35% 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).

ICS-T3 replaces ICS-T2. The new standard has been cross-checked against the old and will give similar results.

\* *The supplied chromatogram shows only one peak. However, it is often found that the two isomers of tetrahydroisocohumulone are partially resolved.*

### Structures of Tetrahydroiso-alpha-acids



Tetrahydroisohumulones & Tetrahydroisoadhumulones  
C<sub>21</sub>H<sub>34</sub>O<sub>5</sub>  
(mw 366.490)

## DCHA-Hexa, ICS-H2

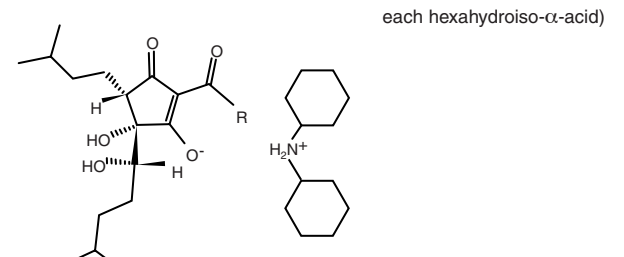
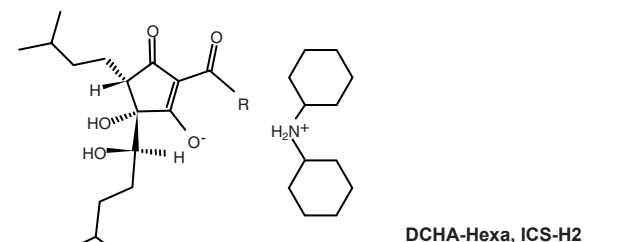
ICS-H2 is a purified preparation of the dicyclohexylamine salts of *cis*-hexahydroiso- $\alpha$ -acids. It is deemed to have a total hexahydroiso- $\alpha$ -acids content of 65.7% (w/w), though this figure takes into account only the major forms of hexahydroiso- $\alpha$ -acids that are present: two *cis*-hexahydroisocohumulones, two *cis*-hexahydroisohumulones and two *cis*-hexahydroisoadhumulones.

If you are using the recommended method, expect the combined areas of the *cis*-hexahydroisocohumulones peaks\* to be about 53% 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).

ICS-H2 replaces ICS-H1. The new standard has been cross-checked against the old and will give similar results.

\* *The supplied chromatogram shows two peaks, corresponding to the two cis forms of this particular iso- $\alpha$ -acid. However, it is not known which peak corresponds to which of the two forms shown above. (The same is also true for the  $\rho$ -isohumulones peaks).*

### Structures of DCHA-Hexahydroiso-alpha-acids



DCHA-hexahydroisohumulones & isoadhumulones  
C<sub>33</sub>H<sub>59</sub>NO<sub>5</sub>  
(mw 549.823)