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International Calibration Standards for HPLC Analysis of Isomerized α-Acids

New International Calibration Standards for HPLC Analysis of Isomerized $\alpha\text{-Acids}$

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 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 α -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 α -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 and Tetra standards (ICS-I2 and ICS-T2) 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.
- 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 α -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 α -acids in all unknown samples.
- Detailed information pertaining to each standard, plus full instruction for use, is available on request and will be 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 α -acid. These standards are therefore recommended primarily for the determination of the total content of isomerized α -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-α-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. American Society of Brewing Chemists (ASBC): See the order form.
- Labor Veritas, Zürich, Engimattstrasse 11 Postfach 353 CH-8027 Zürich, Switzerland.

Phone: (44) 283 29 30 Fax: (44) 201 42 49 E-mail: admin@laborveritas.ch Web site: www.laborveritas.ch

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

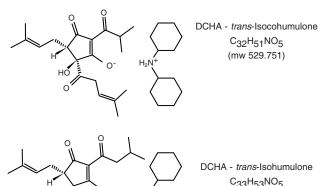
DCHA-Iso, ICS-I2

ICS-I2 is a purified preparation of the dicyclohexylamine salts of *trans*-iso- α -acids. It is deemed to have a total iso- α -acids content of 64.3% (w/w), though this figure takes into account only the major forms of the iso- α -acids that are present: trans-isocohumulone, trans-isohumulone and trans-isoadhumulone.

If you are using the recommended method, expect the area of the trans-isocohumulone peak to be about 47.5% 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).

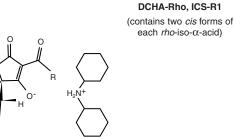
ICS-I2 replaces ICS-I1. The new standard has been cross-checked against the old and will give similar results.

Structures of DCHA-Iso-alpha-acids



DCHA - trans-Isohumulone C33H53NO5 (mw 543.778)

DCHA - trans-Isoadhumulone C33H53NO5 (mw 543.778)



DCHA-rho-isocohumulones DCHA-rho-isohumulones & isoadhumulones C32H53NO5 C33H55NO5 (mw 531.765) (mw 545.791)

Tetrahydroisocohumulones C₂₀H₃₂O₅ (mw 352.464)

HO

cis-

tranc

Tetra, ICS-T2

ICS-T2 is a purified preparation containing both cis- and

tetrahydroisohumulones and tetrahydroisoadhumulones.

If you are using the recommended method, expect the

peak(s)* to be about 39% of the total peak area of all of

may not be the case for methods that use other mobile

phases, or for measurement at different wavelengths).

ICS-T2 replaces ICS-T1. The new standard has a much

small reduction (typically 1 - 2% relative) to the value obtained for "total Tetra" in an unknown sample.

* The supplied chromatogram shows only one peak.

hydroisocohumulone are partially resolved.

Structures of Tetrahydroiso-alpha-acids

However, it is often found that the two isomers of tetra-

higher *cis:trans* ratio than the old and this may result in a

the compounds included in the calibration. (Caution: This

(combined) area of the tetrahydroisocohumulones

In respect of these six isomers it is deemed to have a total

trans-isomers of the tetrahydroisocohumulones,

tetrahydroiso-α-acids content of 99.4% (w/w).

Tetrahvdroisohumulones & Tetrahydroisoadhumulones C₂₁H₃₄O₅ (mw 366.490)

Tetra, ICS-T2

(contains both cis & trans forms of

tetrahydroiso-α-acids)

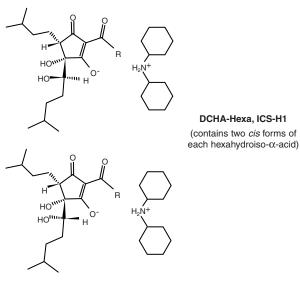
DCHA-Hexa, ICS-H1

ICS-H1 is a purified preparation of the dicyclohexylamine salts of *cis*-hexahydroiso- α -acids. It is deemed to have a total hexahydroiso-a-acids content of 65.7% (w/w), though this figure takes into account only the major forms of hexahydroiso- α -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).

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

Structures of DCHA-Hexahydroiso-alpha-acids



DCHA-hexahydroisocohumulones	DCHA-hexahydroisohumulones
C ₃₂ H ₅₇ NO ₅	& isoadhumulones
(mw 535.796)	С ₃₃ Н ₅₉ NO ₅
х <i>У</i>	(mw 549.823)

Structures of DCHA-rho-Iso-alpha-acids

DCHA-Rho, ICS-R1

acid. However, it is not known which peak corresponds to

ICS-R1 is a purified preparation of the dicyclohexylamine

salts of *cis*- ρ -iso- α -acids. This standard is deemed to have

a total ρ -iso- α -acids content of 65.5% (w/w), though this

figure takes into account only the major forms of the p-

iso- α -acids that are present: two *cis*- ρ -isocohumulones,

two cis-p-isohumulones and two cis-p-isoadhumulones.

If you are using the recommended method, expect the

case for methods that use other mobile phases, or for

* The supplied chromatogram shows two peaks, corre-

sponding to the two cis forms of this particular iso- α -

which of the two forms shown below. (The same is also

measurement at different wavelengths).

true for the p-isohumulones peaks).

HO HO.

HO HO"

combined area of the cis-p-isocohumulones peaks* to be

about 20% of the total peak area of all of the compounds

included in the calibration. (Caution: This may not be the

Total SFr **Fotal** ŝ TOTAL Quantity CHF 250 \$60 \$60 \$60 \$60 ICS-H1 ICS-R1 DCHA-Iso, ICS-I2 Tetra, ICS-T2 DCHA-Hexa, DCHA-Rho,

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Calibration

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 - Phone: (44) 283 29 30 Fax: (44) 201 42 49 E-mail: admin@laborveritas.ch Web site: www.laborveritas.ch
- 2. 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.