

**International Subcommittee for Isomerized Hop α -Acids
Standards**

PRESS RELEASE

**New International Calibration Standard (ICS-I3) for HPLC Analysis of
Iso- α -acids**

(Submitted on behalf of the International Subcommittee for Isomerized Hop α -Acids Standards by Martin Biendl, Vice Chairman).

The International Subcommittee for Isomerized Hop α -Acids Standards (ISIHAS), announces the release of a new calibration standard, ICS-I3, for the HPLC analysis of iso- α -acids. This standard replaces ICS-I2, which is now withdrawn.

Background

In April 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 α -acids in hop products and in beer. Four standards were produced:

- DCHA-Iso, **ICS-I1** (Iso- α -acids standard);
- DCHA-Rho, **ICS-R1** (*Rho*-iso- α -acids standard);
- Tetra, **ICS-T1** (Tetrahydroiso- α -acids standard);
- DCHA-Hexa, **ICS-H1** (Hexahydroiso- α -acids standard).

The purity of each standard was determined using various HPLC procedures, elemental analysis and other methods. In each case, the total content of major isomers and homologs was declared and, before release, the stability of the standard was assessed and recommendations made regarding storage and method of use. An isocratic version of EBC Method 7.8 (now EBC 7.9) was recommended as a convenient and generally applicable HPLC method for use in the analysis of unknown samples containing isomerized or reduced isomerized α -acids.

Over a period of 2½ years, the subcommittee monitored the stocks of the four standards via careful HPLC analysis, finally concluding that the standards had maintained their declared compositions and had not undergone significant chemical change.

Following exhaustion of the original stocks, the “Iso”, “Rho” and “Tetra” standards were replaced by new standards, ICS-I2, ICS-R2 and ICS-T2.

Release of ICS-I3

Stocks of DCHA-Iso (ICS-I2) are now almost exhausted. In anticipation of this, in 2007 the subcommittee initiated the preparation of a replacement standard. The new standard was prepared in the laboratories of Kalsec, Inc., under the guidance of subcommittee member, Louis Burroughs. Packaging was also carried out in the same laboratory. The subcommittee records its considerable appreciation to Louis Burroughs and his colleagues for all their skilled input to the successful manufacture of this new standard. Following preparation, subcommittee members conducted extensive analysis in order to validate the new standard and assign a value to the content of "Iso" isomers, this work including a collaborative HPLC study in which the prospective new standard was crosschecked against the existing standard.

This new standard, ICS-I3, now 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 ICS-I3 is not identical to the standard it replaces, but it can be used in exactly the same way. Dependent upon circumstances, users may find that the results for an unknown sample may differ slightly according to whether the old or the new standard has been used. In most cases though, the differences observed when quantifying iso- α -acids using ICS-I3 instead of ICS-I2 will be found to be very slight. The magnitude of this shift will vary according to the actual isomeric (*cis-* and *trans-*) and homological (co-, n- and ad-) composition of the unknown sample, but can be expected to be usually within the normal range of experimental error. ICS-I3 has a substantially lower ratio of co-isohumulone than its predecessor and better reflects the ratios found in commercially available Iso products.

How to Purchase

Stocks of ICS-I3 are being divided between ASBC (in USA) and Labor Veritas (in Switzerland), from which sources analysts can purchase the new standard in the usual 250mg vials.* Orders are dispatched by express mail to minimize risk of damage in transit.

Detailed information pertaining to each standard, including full instructions for use, is available from ASBC or Labor Veritas on request and is automatically supplied with each purchase. ICS-I2 will now be available only while stocks last.

** Purchasers in the USA, Canada, Central & South America and Japan should contact ASBC headquarters (email: asbc@scisoc.org; tel: +1 (651) 454-7250), while those in Europe and Africa should direct enquiries to Labor Veritas, Zürich, Switzerland (email: admin@laborveritas.ch; tel: +41 (0) 44 283 29 30). Persons ordering from other parts of the world may make their approach to either party).*



MATERIAL SAFETY DATA SHEET



International Calibration Standard: ICS-I3 (“DCHA-Iso”)

Section I. PRODUCT AND SUPPLIERS	
Supplier's Name 1. ASBC 2. Labor Veritas	Emergency Telephone Nos. 1. USA: (+1) 651 454 7250 or 2. Switzerland: (+41) (0) 44 283 29 30
Address 1. ASBC, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA or 2. Labor Veritas, Engimattstrasse 11, Postfach 353, CH-8027 Zürich, Switzerland	
Product Name ICS-I3	Synonyms DCHA-Iso; Iso Standard
CAS No(s). Not available	Empirical Formula Contains mainly C ₃₂ H ₅₁ NO ₅ & C ₃₃ H ₅₃ NO ₅
Chemical Family Isomerized hop resin acid	Molecular Weight Mainly 530 & 544 daltons

Section II. COMPOSITION	
Material or components	%
A purified, semi-crystalline preparation of <i>trans</i> -iso- α -acids in dicyclohexylamine (“DCHA”) salt form. Derived from CO ₂ extracted and isomerized α -acids of hops. Mostly contains co-, n- and ad-homologs.	97% DCHA salts of <i>trans</i> -iso- α -acids (DCHA content: approx. 34%)

Section III. PHYSICAL DATA	
Boiling Point °C (°F.) Not applicable	Solubility in Water Slightly soluble; soluble at pH 8 -11
Melting Point °C (°F.) 168°C (334°F)	Solubility in Alcohol Soluble
Vapor Pressure (mm Hg.) Not applicable	Specific Gravity (H₂O = 1) Not applicable
Vapor Density (AIR =1) Not applicable	Volatiles by volume (%) Not applicable
	Evaporation Rate (Ether =1) Not applicable
Appearance and Odor A white, virtually odorless, semi-crystalline powder.	

Section IV. FIRE AND EXPLOSION HAZARD DATA			
Flash Point (Method used) Not applicable	Flammable Limits Not applicable	Le¹	Ue^f
Extinguishing Media Water, CO ₂ , Foam, Dry Powder			
Specific Fire Fighting Procedures None required.			
Unusual Fire and Explosion Hazards None known			

Section V. HEALTH HAZARD DATA
Important Note This product is intended for use only as an HPLC calibration standard! DO NOT add to food, water or beer that may be tasted or consumed by humans!
Threshold Limit Value None known
Effects of Exposure No data available. May be toxic; possible carcinogen. <u>Oral Ingestion</u> : Not known; ingestion of large amounts is probably dangerous. <u>Eye Contact</u> : Believed to be irritant; may cause corneal inflammation. <u>Skin Contact</u> : Prolonged contact may possibly cause dermatitis in some individuals. <u>Respiratory Tract</u> : Probably irritant or corrosive.
Emergency and First Aid Procedures <u>Eye contact</u> : Wash with copious amounts of water; seek medical attention. <u>Skin contact</u> : Remove with warm soapy water.

Section VI. SPILL OR LEAK PROCEDURES
Scoop or vacuum material into disposal container. Clean area with disposable cloth, warm water and detergent to remove final traces.

Section VII. SPECIAL PROTECTION & PRECAUTION INFORMATION	
Respiratory Protection (Specify type) Simple dust mask recommended. Avoid raising dust.	
Ventilation Normally, none required.	
Protective Gloves Laboratory grade, flexible latex or vinyl gloves recommended.	Eye Protection Protective goggles or safety glasses recommended.
Other Protective Equipment Use of low-grade protective clothing (e.g. laboratory coat) recommended.	

Precautions Taken in Handling and Storing

Store in freezer in original container. Protect from moisture.

Minimum storage temperature: -20°C (-4°F)

Maximum storage temperature (to retain as a standard): -10°C (14°F)

Maximum storage temperature (for safety purposes only): 30°C (86°F)

Disclaimer

The information given in this MSDS is believed correct and is supplied in good faith but without warranty of any kind. It should be used only as a supplement to information already in your possession concerning this product and its use. The determination of whether and under what conditions you or your employees should handle and use the product is yours to make.



International Calibration Standards for HPLC Analysis of Isomerized & Reduced Isomerized α -Acids



DCHA-Iso, ICS-I3 (Iso- α -acids standard)
DCHA-Rho, ICS-R2 (*Rho*-iso- α -acids standard)
Tetra, ICS-T2 (Tetrahydroiso- α -acids standard)
DCHA-Hexa, ICS-H1 (Hexahydroiso- α -acids standard)

History

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 beers. The resultant **International Calibration Standards (ICS)** were produced, analyzed, and verified for the benefit of the brewing industry by the International Sub-committee for Isomerized Hop α -Acids Standards and first released in 2001, since which time three of the original standards have now been superseded by new preparations.

Descriptions

- The four current standards are crystalline preparations containing *trans*-iso- α -acids (ICS-I3); *cis-p*-iso- α -acids (ICS-R2); *cis*- & *trans*- tetrahydroiso- α -acids (ICS-T2) and *cis*-hexahydroiso- α -acids (ICS-H1). Used as instructed, they are considered suitable for totaled, quantitative analysis of all normally encountered mixtures of *cis* & *trans* isomers and major homologs (co, n & ad forms).
- Excepting in the case of ICS-T2, these standards are presented in the form of their dicyclohexylamine (DCHA) salts. Their purities were initially measured using a variety of HPLC procedures, agreement between these different methods being considered acceptable.
- The standards were later re-analyzed using an isocratic version of EBC Method 7.8 (now issued as EBC Method 7.9), following which their total content of the major isomerized or reduced isomerized α -acids were determined as being:

DCHA-Iso, ICS-I3: **62.3%** (of which *trans*-isocohumulone = ~ 33.6% by relative peak area)

DCHA-Rho, ICS-R2: **65.3%** (of which *cis-p*-isocohumulones = ~ 14.5% by relative peak area)

Tetra, ICS-T2: **99.4%** (of which *cis*- & *trans*- tetrahydroisocohumulones = ~ 39% by relative peak area)

DCHA-Hexa, ICS-H1: **65.7%** (of which *cis*-hexahydroisocohumulones = ~ 53% by relative peak area)

- **Instructions for use are supplied. For peak identification purposes, these include typical chromatograms of the standards themselves, as obtained by the recommended HPLC method,**
- Comparative results from an 2-year stability test of standards held at freezer and room temperature indicated excellent stability under either temperature, although freezer storage is strongly recommended. (*The composition of these standards, as held under recommended storage conditions, will be monitored to ensure that their purity is maintained.*)

Limitations

Most HPLC methods will not reliably separate (if present) all *cis*- and *trans*- isomers of the three, major homologs (co, n & ad forms) of a given type of isomerized α -acid. These standards are therefore recommended primarily for the determination of the **total** isomerized α -acids of the stated type in an unknown sample - though it may often be possible to concurrently determine the approximate cohumulone homologs content.

Furthermore, 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 different types of iso- α -acids are present together in an unknown sample (e.g. iso- α -acids plus p-iso- α -acids), then these may not be fully resolved by some HPLC methods, leading to possible errors of identification and hence of quantitative evaluation.

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