

Save the Flavor — Robust Iso- α -Acids Assaying in Beer within Ten Minutes

Michael Heidorn
Thermo Fisher Scientific, Germering, Germany

Key Words

Beer, Bitterness, Bitter Substances, Isohumulones, Fingerprint, Characteristic Pattern, *Cis*- and *Trans*-isomerism

Goal

Determination of the beer bitterness by measuring the contents of isohumulones in untreated beer – quickly, including sample preparation, HPLC separation, and data review. Furthermore, the application needs to be robust and must provide reproducible results, increased lifetime of the analytical column, and provide a fingerprint or characteristic pattern of the *cis*- and *trans*-ratios.



Introduction

Isohumulones (iso- α -acids) are derived by humulones (α -acids), essential constituents of hop resins. The poorly water-soluble α -acids are isomerized to the better water-soluble iso- α -acids during wort-boiling. Iso- α -acids form approximately eighty percent of the typical bitterness of beer. Their antimicrobial effect leads to a sterile beverage, their tensioactive character stabilizes the foam, and they have a major influence on the general flavor, smell, and smoothness of beer.¹ The three major iso- α -acid variants which are basically present in beer only differ in their acyl side chain and comprise iso-n-humulone, iso-cohumulone, and iso-adhumulone. Due to the stereochemistry of iso- α -acids, all of them occur as *cis*- and *trans*-isomer (Figure 1).

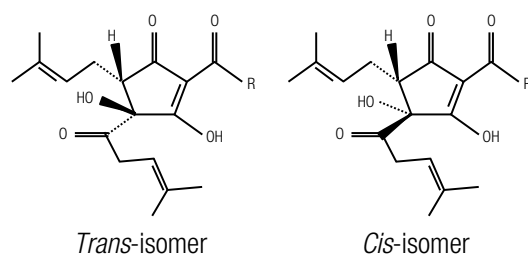


Figure 1: Chemical structure of *cis*- and *trans*-iso- α -acids.

<i>Trans</i> -isomer	<i>Cis</i> -isomer
R = CH(CH ₃) ₂	Iso-cohumulone
R = CH ₂ CH(CH ₃) ₂	Iso-n-humulone
R = CH(CH ₃)CH ₂ CH ₃	Iso-adhumulone

Each iso- α -acid variant provides different contributions to beer taste and foam stability. Recent investigations have shown that these differences are even true between both *cis*- and *trans*-isomers of the same iso- α -acid.¹ Furthermore, the lifetimes of *cis*- and *trans*-isomers significantly differ from each other. Degradation products of iso- α -acids sensitively influence the important beer attributes mentioned above and the avoidance of less stable iso- α -acid variants is beneficial.²

Precise as well as comparable information about the genuine beer bitterness is only achievable by specific quantitation of bitter substances (isohumulones) in beer. Furthermore, the fingerprint or characteristic pattern of the *cis*- and *trans*-ratios is very important information due to the reasons mentioned above. High-Performance Liquid Chromatography (HPLC) is the only analytical method that provides these results. If injected untreated, beer causes reproducibility issues and compromises its lifetime of the column as beer does not only consist of isohumulones but also of a very complex matrix. Manual beer sample pretreatment steps, like off-line solid phase extraction (SPE) are commonly used but are very time consuming. Furthermore, traditional HPLC analyses last about half an hour.

Thermo
SCIENTIFIC

Experiment

Equipment: System package with on-line SPE RS configuration (P/N 5200.0500) and isohumulones starter kit for on-line SPE RS system (P/N TS-MKIT0012).

Samples: Beer (German Pilsner, purchased from a local grocery store) and certified isohumulones standard DCHA-Iso, ICS-I3 (part of P/N TS-MKIT0012).

Conditions: Experimental data: listed in Figure 2.

Figure 2: Chromatogram of isohumulones in beer and isohumulones standard (overlay).

Results and Discussion

This application provides an instant result about the content of isohumulones in untreated beer within less than ten minutes, including sample preparation, HPLC separation, and result evaluation. Sample preparation runs automatically and is not prone to manual errors resulting in highest reproducibility. Since no manual, time-consuming labor is needed, potential health risks are reduced. Samples can be run unattended, for instance, overnight or over the weekend which results in increased workload per system and, therefore, higher returns on investment. Furthermore, the HPLC separation is very robust and provides very reproducible results next to an increased lifetime of the analytical column. Being the most important benefit, the specific fingerprint or characteristic pattern of the *cis*- and *trans*-ratios is fully explored at each time for each beer.

Conclusion

The application shown here provides specific determination and quantitation of each *cis*- and *trans*-isomer of the isohumulones (iso- α -acids) within a single run. Since isocratic conditions are applied, the HPLC system is always in steady state and the analytical run is finished within ten minutes – from sampling to result. By using online SPE, an untreated beer sample is injected directly, all SPE-steps are performed automatically, and the entire analysis lasts only nine minutes. Thereby, the application represents perfect and easy-to-use beer quality monitoring. The UltiMate 3000 RS System Package with On-Line SPE in combination with the Isohumulones Starter Kit for On-Line SPE RS System provide all instrument hardware, software, and consumables needed to run this application. The certified isohumulones standard DCHA-Iso, ICS-I3 can be used for identification or calibration of the individual isohumulones.

System: Thermo Scientific™ Dionex™ UltiMate™ 3000 System with On-Line SPE RS Configuration
Mobile Phase: A – water with 1% formic acid and 100 mg/L ethylenediaminetetraacetic acid disodium salt dihydrate
B – acetonitrile
Pressure: 720 bar (max.)
Temperature: 35 °C
Injection: 15 μ L beer or 5 μ L isohumulone standard
Analytical Flow Path Parameters
Column: Thermo Scientific™ Hypersil GOLD™ column, 1.9 μ m, 100 \times 2.1 mm
Isocratic: 50% B
Flow Rate: 650 μ L/min
Detection: Thermo Scientific Dionex UltiMate VWD-3400RS Variable Wavelength Detector, 2.5 μ L flow cell, 270 nm
Automated On-Line SPE Parameters
Column: Hypersil GOLD C8 column, 5 μ m, 20 \times 2.1 mm
Gradient: 0-2 min 25% B at 2000 μ L/min,
2-4 min 100% B at 2000 μ L/min,
4-7 min 25% B at 200 μ L/min,
7-9 min 25% B at 2000 μ L/min
Valve Position: 0 min 6_1, 1.5 min 1_2, 2 min 6_1

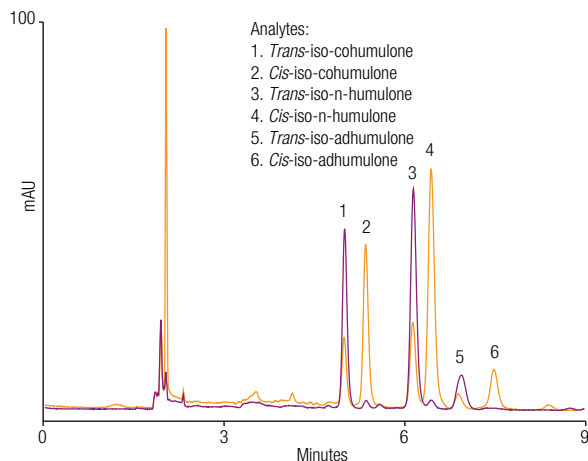


Figure 2: Chromatogram of isohumulones in beer and isohumulones standard (overlay).

References

1. Caballero, I., Blanco, C. A., and Porras, M. Iso- α -Acids, bitterness and loss of beer quality during storage. *Trends Food Sci. Technol.* (online). 2012. <http://dx.doi.org/10.1016/j.tifs.2012.01.001>.
2. Heidorn, M. Fast Determination of Iso-alpha-Acids in Untreated Beer Samples with Specific Separation into *cis*- and *trans*-Isomers. *J. Am. Soc. Brew. Chem.* (online). 2013. <http://dx.doi.org/10.1094/ASBCJ-2013-0506-01>.
3. Thermo Scientific Application on Brief 155: Monitor the Brewing Process with LC-Transformation of Hop α -Acids into Beer Iso- α -Acids. Germering, Germany, 2014. (online) <http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD Documents/Application & Technical Notes/AB155-Monitor-Brewing-Process-HPLC-AB70981-E.pdf> (accessed March 3, 2014)
4. Thermo Scientific Application Brief 156: The Everlasting Paradigm-Keep Beer Tradition or Prevent Beer from a Skunky Off-Flavor?. Germering, Germany, 2014. (online) <http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD Documents/Application & Technical Notes/AB156-Everlasting-Paradigm-Beer-Tradition-AB70982-E.pdf> (accessed March 3, 2014).

Useful Links

- Heidorn, M., Martin, M. M., Bauder, R., and Steiner, F. Effective On-Line Sample Clean-Up and analyte Enrichment for UHPLC Analyses. Poster Note 70170_E 06/12S. http://www.dionex.com/en-us/webdocs/113647-PN-LC-Beer-SPE-UHPLC-22Jun2012-70170_E.pdf
- Thermo Scientific Isohumulones in Beer Application Kits <http://www.thermoscientific.com/ecomm/servlet/newsdetail?storeId=11152&contentId=57083>
- UHPLC Solutions Unveil Crucial Secrets in Beer Flavor (1) <http://chromblog.thermoscientific.com/blog/bid/100399/UHPLC-Solutions-Unveil-Crucial-Secrets-in-Beer-Flavor-1>
- UHPLC Solutions Unveil Crucial Secrets in Beer Flavor (2) <http://chromblog.thermoscientific.com/blog/bid/100401/UHPLC-Solutions-Unveil-Crucial-Secrets-in-Beer-Flavor-2>
- UHPLC & On-Line SPE Speeds Up Analysis of Isohumulones in Beer <http://chromblog.thermoscientific.com/blog/bid/88460/UHPLC-On-Line-SPE-Speeds-Up-Analysis-of-Isohumulones-in-Beer>

www.thermofisher.com/dionex

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Africa +43 1 333 50 34 0	Denmark +45 70 23 62 60	Japan +81 6 6885 1213	Russia/CIS +43 1 333 50 34 0
Australia +61 3 9757 4300	Europe-Other +43 1 333 50 34 0	Korea +82 2 3420 8600	Singapore +65 6289 1190
Austria +43 810 282 206	Finland +358 9 3291 0200	Latin America +1 561 688 8700	Sweden +46 8 556 468 00
Belgium +32 53 73 42 41	France +33 1 60 92 48 00	Middle East +43 1 333 50 34 0	Switzerland +41 61 716 77 00
Brazil +55 11 3731 5140	Germany +49 6103 408 1014	Netherlands +31 76 579 55 55	Taiwan +886 2 8751 6655
Canada +1 800 530 8447	India +91 22 6742 9494	New Zealand +64 9 980 6700	UK/Ireland +44 1442 233555
China 800 810 5118 (free call domestic) 400 650 5118	Italy +39 02 950 591	Norway +46 8 556 468 00	USA +1 800 532 4752

Thermo
SCIENTIFIC

Part of Thermo Fisher Scientific