

# Monitor the Brewing Process with LC– Transformation of Hop $\alpha$ -Acids into Beer Iso- $\alpha$ -Acids

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## Keywords

Humulones,  $\alpha$ -Acids, Isohumulones, *cis*- and *trans*-isomerism, Beer Bitterness, Bitter Acids, Bittering Agents, Wort Boiling, Lupulones

## Goal

Within a single run, each humulone and all *cis*- and *trans*-isomers of the isohumulones are detected—as fast as possible. The beer is injected untreated without compromising the analytical result in regard to resolution, retention, reproducibility, or by shortening the analytical column lifetime.

## Introduction

Since ancient times, hop (*Humulus lupulus* L.) has been a main ingredient of beer. Some of the most essential constituents of hop resins are the humulones ( $\alpha$ -acids) which are poorly water soluble. During wort boiling, they are isomerized to better water soluble isohumulones (iso- $\alpha$ -acids). These isohumulones mainly comprise iso-n-humulone, isochumulone, and iso-*adhumulone*. Due to their stereochemistry, each of them occurs as a *cis*- and a *trans*-isomer (Figure 1).

Monitoring the isomerization progress as well as the general content of iso- $\alpha$ -acids in beer during and after the brewing process is mandatory in order to control important beer properties: each iso- $\alpha$ -acid variant



provides different contributions to beer bitterness, taste and foam stability—and they even exhibit different lifetimes. Since the degradation products of iso- $\alpha$ -acids also influence the beer attributes mentioned above, the avoidance of hop types containing much of the less stable  $\alpha$ -acid variants is beneficial in terms of the stability of the beer quality characteristics. Recently, it was proven that these differences are already true between both *cis*- and *trans*-isomers of the same iso- $\alpha$ -acid.<sup>1</sup>

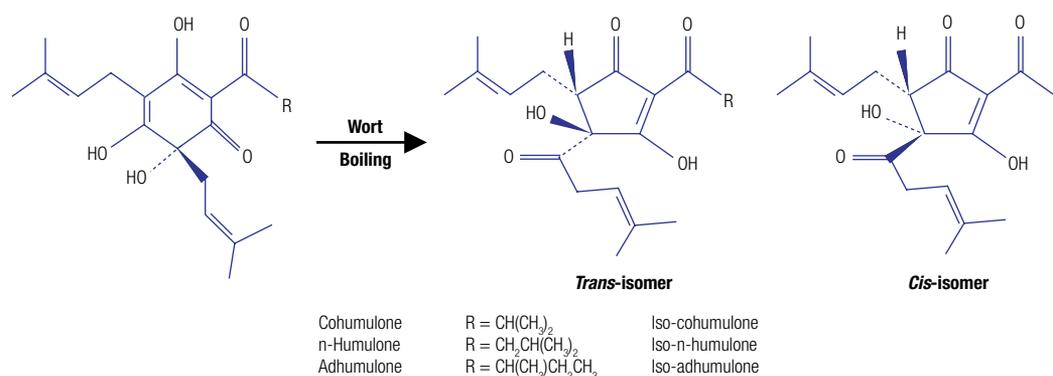


Figure 1. Transformation of  $\alpha$ -acids to *cis*- and *trans*-iso- $\alpha$ -acids during wort-boiling.

HPLC is the only method that ensures separation of isohumulones from humulones. When untreated, wort or beer is directly injected on an analytical column, where the very complex sample matrix can cause reproducibility issues and also compromise column lifetime.<sup>2</sup> Hence, manual sample pretreatment steps like off-line solid phase extraction (SPE) are often used. Traditional HPLC analyses last about three-fourths of an hour when analyzing humulones next to isohumulones in beer. Since manual SPE is very time-consuming, an entire analysis of a single beer sample lasts at least an hour from sampling to result—and in the majority of cases, even longer.

## Experimental

### 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).

### Sample

Humulones and lupulones standard (P/N TS-7003002) mixed with German Pilsener beer.

### Conditions

Experimental data is listed in Figure 2.

## Results and Discussion

Each humulone and all of the *cis*- and *trans*-isomers of the isohumulones were detected in less than ten minutes, making this application a powerful tool for process monitoring. In addition, the received specific pattern of the humulones, as well as *cis*- and *trans*-isohumulones ratio, tells the expert brewer about the beer bitterness and properties to expect. Furthermore, lupulones (hop  $\alpha$ -acids, in particular colupulone, n-lupulone, and adlupulone) elute after thirteen minutes. Lupulones do not isomerize during wort boiling, are poorly water soluble, have a negligible effect on the general beer taste, but contribute harsh beer bitterness if at all present in beer. Nevertheless, the entire analysis lasts fifteen minutes including sample preparation, LC separation, system re-equilibration, and result evaluation. Sample preparation runs automatically by applying automated on-line SPE. This leads to highest reproducibility and reduction of potential health risks as manual handling errors are precluded. Furthermore, time-consuming labor is also reduced and 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. The LC separation is very robust and provides very reproducible results as well as an increased lifetime of the analytical column. The isomerization progress can be observed or beers are easily screened for remaining humulones (or lupulones). Filter syringes are highly recommended for the analysis of wort prior to injection onto the SPE column.

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: 750 bar (max.)  
 Temperature: 35 °C  
 Injection: 15  $\mu$ L

### Analytical Flow Path Parameters

Column: Thermo Scientific™ Hypersil GOLD™, 1.9  $\mu$ m, 100  $\times$  2.1 mm  
 Gradient: 0–4 min 50% B, 4–6 min 50–60% B, 6–7 min 60% B; 7–8 min 70% B, 8–11.5 min 70–80% B; 11.5–15 min 50% B  
 Flow Rate: 650  $\mu$ L/min  
 Detection: WWD-3400RS, 2.5  $\mu$ L flow cell, 270 nm

### Automated On-Line SPE Parameters

Column: Hypersil Gold C8, 5  $\mu$ m, 20  $\times$  2.1 mm  
 Gradient: 0–2.5 min 25% B at 2000  $\mu$ L/min, 2.5–5 min 100% B at 2000  $\mu$ L/min, 5–14 min 25% B at 200  $\mu$ L/min, 14–15 min 25% B at 2000  $\mu$ L/min  
 Valve Position: 0 min 6\_1, 1.5 min 1\_2, 2 min 6\_1

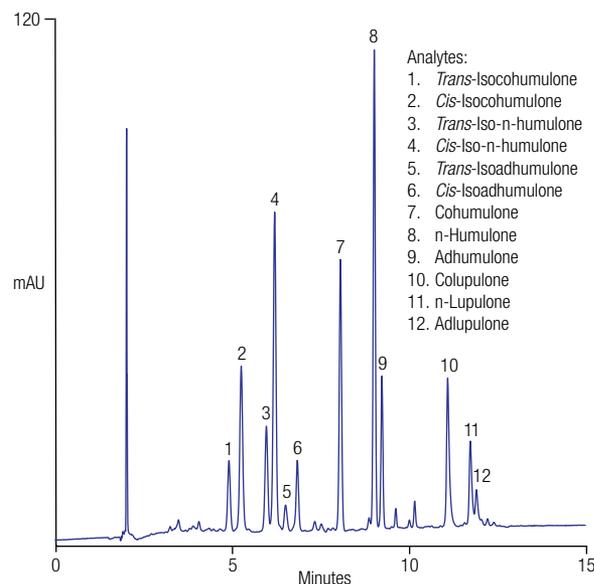


Figure 2. Chromatogram of hop bitter acids and iso- $\alpha$ -acids in beer.

## Conclusion

The application shown here provides specific determination and quantitation of each  $\alpha$ -acid as well as each *cis*- and *trans*-isomer of the iso- $\alpha$ -acids within a single run. By applying online SPE, an untreated beer sample is injected directly, all SPE-steps are performed automatically, and the entire analysis lasts only fifteen minutes, while getting the essential result after nine minutes. The UltiMate 3000 RS System Package with On-Line SPE and the Isohumulones Starter Kit for On-Line SPE RS System provide all instrument hardware, software, and consumables needed to run this application. A ready-to-use, certified humulones and lupulones standard can also be purchased from Thermo Scientific.

## References

1. Caballero, I., Blanco, C. A., and Porras, M. Iso- $\alpha$ -Acids, Bitterness and Loss of Beer Quality During Storage. *Trends Food Sci. Technol*, 2012 [Online] <http://dx.doi.org/10.1016/j.tifs.2012.01.001>
2. 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 [Online] [http://www.dionex.com/en-us/webdocs/113647-PN-LC-Beer-SPE-UHPLC-22Jun2012-70170\\_E.pdf](http://www.dionex.com/en-us/webdocs/113647-PN-LC-Beer-SPE-UHPLC-22Jun2012-70170_E.pdf)

## Useful Links

Thermo Scientific Application Brief 153: Save the Flavor—Robust Iso- $\alpha$ -Acids Assaying in Beer within Ten Minutes. Germering, Germany, 2013. [Online] <http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/AB-153-Robust-Iso-alpha-Acids-Assaying-Beer-Ten-Minutes-AB70882-E.pdf> (accessed Feb 14, 2014).

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%20Documents/Application%20&%20Technical%20Notes/AB156-Everlasting-Paradigm-Beer-Tradition-AB70982-E.pdf> (accessed March 3, 2014).

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*, 2013 [Online] <http://dx.doi.org/10.1094/ASBCJ-2013-0506-01>

Thermo Scientific Isohumulones in Beer Application Kits <http://www.thermoscientific.com/ecommservlet/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>

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