The Everlasting Paradigm—
Keep Beer Tradition or Prevent Beer from a Skunky Off-Flavor?

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Key Words
Reduced Isohumulones, Light-Struck, German Purity Law, Isohumulones, cis- and trans-isomerism, Beer, Brewing process, Total Solution for Isohumulones in Beer

Goal
Within a single run, each cis- and trans-isomer of the isohumulones or reduced isohumulones shall be detected as fast as possible—from sampling to result. The cis- and trans-ratios shall be fully receivable. The beer shall be injected untreated but without compromising the analytical result in regard to resolution, retention, reproducibility, or by shortening the analytical column lifetime.

Introduction
Typical and important characteristics of beer bitterness are provided by isohumulones (iso-α-acids) which mainly comprise iso-n-humulone, isocohumulone, and isoadhumulone. They also have a major influence on the general flavor, aroma, and smoothness of beer and its foam.1 Lifetimes of isohumulone variants significantly differ from each other. Their degradation products sensitively influence the important beer attributes mentioned above; e.g., iso-α-acids decompose to 3-methyl-2-butene-1-thiol (MBT) when exposed to sunlight. MBT is detectable by odor and taste, generally known as skunky off-flavor or light-struck beer, at the extremely low threshold of 0.05 ppb.

Isohumulones can be reduced by synthetic hydrogenation (Figure 1). Such reduced isohumulones do not decompose to MBT and are often used for beers which are filled into clear or green bottles. On the other hand, the German purity law (Reinheitsgebot) requires that only malted grains, hops, yeast and water have to be used—each in an all-natural, untreated state. Synthetically modified ingredients are not allowed to be used during or after the beer brewing process.

![Figure 1. Transformation of iso-α-acids to tetrahydro-iso-α-acids.](image-url)
HPLC is the only method that ensures the separation of isohumulones from their reduced relatives. If untreated beer is directly injected on an analytical column, the very complex sample matrix can cause reproducibility issues and also compromise column lifetime. Manual beer sample pretreatment steps like off-line solid phase extraction (SPE) are commonly used. Furthermore, traditional HPLC analyses last about half an hour when analyzing reduced isohumulones in beer. Since manual SPE is very time consuming, an entire analysis of a single beer sample can last an hour from sampling to result—or 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:* German Pilsener beer spiked with tetrahydro-isohumulones standard (P/N TS-7005002).

*Experimental data:* Listed in Figure 2.

**Results and Discussion**

This application provides an instant result about the content of isohumulones or their reduced counterparts in untreated beer in less than ten minutes, including sample preparation, LC separation, and result evaluation. Since a mobile phase gradient is applied, additional five minutes are used for the equilibration of the analytical and SPE column. 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 next to an increased lifetime of the analytical column. Beer samples can easily be screened for reduced isohumulones if they are meant to comply with the German purity law. Quite contrary, beers or extracts of reduced isohumulones can be screened for residual (non-reduced) isohumulones when the light-struck flavor might become an issue, e.g., if the beer is wanted to be filled in clear or green bottles.

![Figure 2. Chromatogram of iso-α-acids and tetrahydro-iso-α-acids in beer.](image-url)
Conclusion

The application shown here provides specific determination and quantitation of each cis- and trans-isomer of the iso-α-acids and/or reduced iso-α-acids within a single run. Since the analytical result is obtained within ten minutes, this application represents a perfect method for monitoring beer quality. By using on-line SPE, an untreated beer sample is injected directly, all SPE-steps are performed automatically, and the entire analytical procedure lasts only fifteen minutes. 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. A ready-to-use, certified tetrahydro-isohumulones standard can also be purchased from Thermo Fisher Scientific.

References


Useful Links


Thermo Scientific Isohumulones in Beer Application Kits http://www.thermoscientific.com/ecomm/servlet/newsdetails?storeId=11152&contentId=57083


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