Analyse quantitative : quality control of beer

Sonja Schneider. Agilent Technologies, Inc. Waldbronn, Germany. ***On-site Quality Control of Beer*.** Quantification of Isohumulones and Reduced Isohumulones Using the Agilent 1220 Infinity Mobile LC Solution. ***(Extraits)***

Introduction

This Application Note shows the analysis of isohumulones and reduced isohumulones (*trans*-tetrahydro-iso-a-acids) in different types of beer. […] Hop (*Humulus lupulus*) is an herbaceous climbing plant in the family of *Cannabinaceae*. The hop cones contain the bitter *alpha* and *beta* acids: humulones, cohumulones, and adhumulones (*alpha*) and lupulones (*beta*). The humulones are thermally isomerized during the brewing process (Figure 1) leading to higher solubility and more intensive bitterness. After isomerization, the acids result in three pairs of *cis*/*trans* isomerized a-acids, differing in their side chains: *cis*/*trans*-isocohumulones, *cis*/*trans*-isohumulones, and *cis*/*trans*-isoadhumulones.
Isohumulones contribute highly to the typical beer flavor, for example, the bitter taste, with concentrations varying between 5 and 100 ppm. Additionally, they have bacteriostatic properties and perform an important function in foam stability. […]

The bitterness in beers is measured in IBU, defined from the European Brewery Convention (EBC), in which 1 IBU equals 1 mg of dissolved iso-a-acid per L. Bitterness is traditionally analyzed using spectrometric analyses. However, this analysis is limited due to its inability to distinguish the sources of bitterness. High performance liquid chromatography (HPLC) with ultraviolet (UV) detection has become a standard method for the determination of isohumulones 6,7. […]

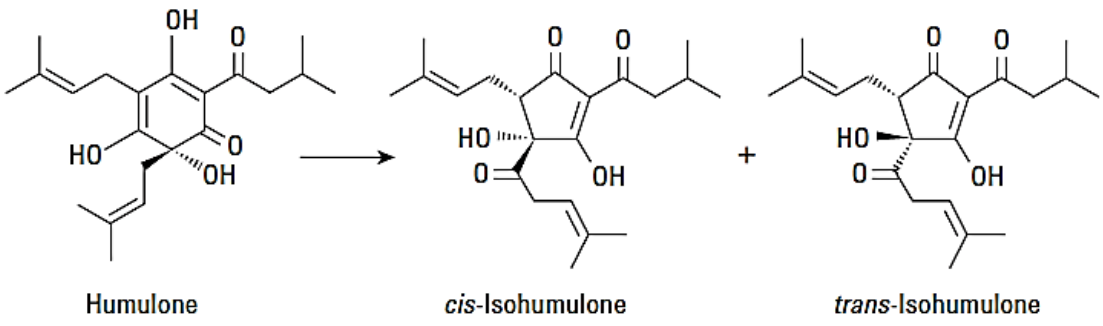


Figure 1. Thermal isomerization of humulones to isohumulones during the brewing process.

[…]

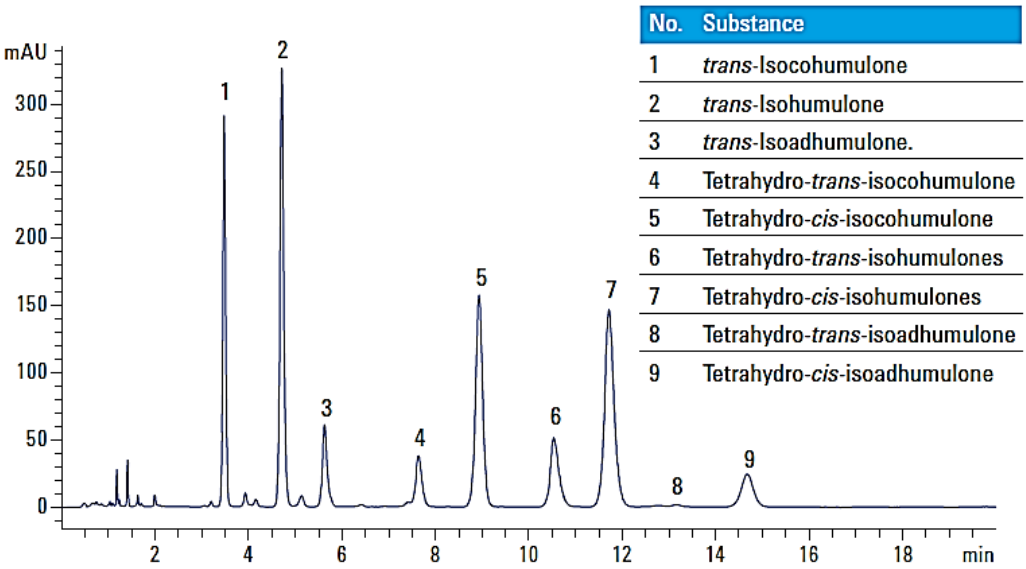
Results and Discussion

Isohumulone standards were separated isocratically using the mobile phase described in Table 1. All nine peaks were well separated (Figure 2). The addition of EDTA ensured optimal peak shape and, therefore, improved resolution in comparison to eluents without EDTA (data not shown). Three nonreduced *trans*-isohumulone and six reduced isohumulone (both *cis* and *trans*-isomers) standards were used for the evaluation of precision and linearity. […]

Table 1. Chromatographic conditions.

Chromatographic conditions	
Solvent	ACN:H_2O + H_3PO_4 to pH 2.8 (52:48, v/v) + 1 mL EDTA 0.1 M/L solvent
Flow rate	1.8 mL/min
Stoptime	20 minutes
Injection volume	5 µL (standards) or 20 µL (beer samples) injection with needle wash
Temperature TCC	35 °C
DAD	270 nm/4 nm, Ref.: OFF
Peak width	> 0.025 minutes (0.5 seconds response time) (10 Hz)

[…]



No.	Substance
1	*trans*-Isocohumulone
2	*trans*-Isohumulone
3	*trans*-Isoadhumulone.
4	Tetrahydro-*trans*-isocohumulone
5	Tetrahydro-*cis*-isocohumulone
6	Tetrahydro-*trans*-isohumulones
7	Tetrahydro-*cis*-isohumulones
8	Tetrahydro-*trans*-isoadhumulone
9	Tetrahydro-*cis*-isoadhumulone

Figure 2. Separation of nine isohumulone standards, nonreduced (Peaks 1–3) and reduced (Peaks 4–9) with isocratic elution.

[…]

Conclusion

Isohumulone standards and isohumulones in 14 beer samples (top-and bottom-fermented) were qualitatively and quantitatively analyzed using the Agilent 1220 Infinity Mobile LC Solution. A simple analytical setup with direct injection (without SPE*) and isocratic elution** allows less experienced users to perform isohumulone analysis in beer. The analysis of the nonreduced and reduced isohumulones was highly precise and linear with correlation coefficients over 0.999 %. The IBUs were calculated, and, as expected, significant differences were found from weizen beer to pils. In most of the beer types, nonreduced isohumulones were detected except for the American premium lager, which contained only reduced isohumulones.

The 1220 Infinity Mobile LC Solution, is a robust and rugged system that enables easy on-site measurement of isohumulones in beer in a simple analytical setup. […]

* SPE : extraction en phase solide (solid phase extraction).

** Isocratic elution : une élution isocratique est une élution au cours de laquelle la composition de la phase mobile n'est pas modifiée au cours du temps, par opposition à une élution par gradient au cours de laquelle la composition de la phase mobile est modifiée en continu ou par paliers.