On-site Quality Control of Beer

Quantification of Isohumulones and Reduced Isohumulones Using the Agilent 1220 Infinity Mobile LC Solution

Application Note

Food Testing & Agriculture

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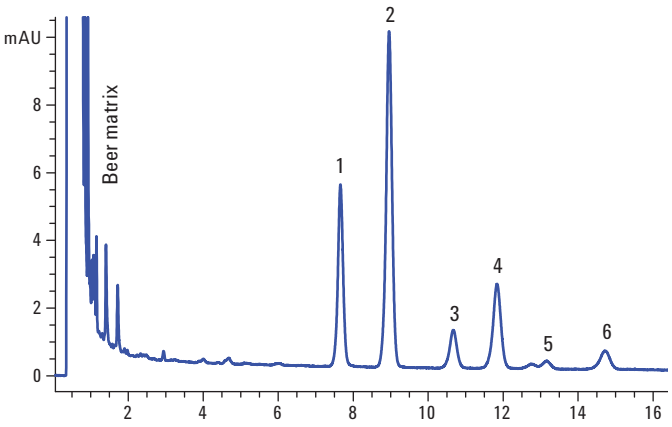
Abstract

This Application Note shows the analysis of isohumulones in different beer samples using the Agilent 1220 Infinity Mobile LC Solution. Direct injection of beer samples and isocratic elution mode enables the user to perform easy on-site measurements in a mobile laboratory without time-consuming sample preparation or method development. With this setup, nonreduced and reduced isohumulone standards were analyzed with high precision and linearity. Isohumulones were determined and quantified in 14 different beer samples. The International Bitterness Units (IBU) were calculated for the tested beer samples revealing significant differences from, for example, lager beer to pils. Reduced isohumulones could only be detected in one beer sample, an American lager beer.



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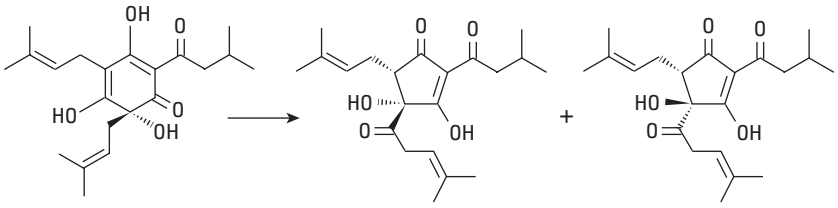
Introduction

This Application Note shows the analysis of isohumulones and reduced isohumulones (*trans*-tetrahydro-iso-α-acids) in different types of beer. Beer is one of the most popular and widely consumed beverages in the world, with an average annual beer consumption of over 100 L per head in Germany, for example[1]. Beer is an alcoholic beverage produced by fermentation of the basic ingredients, water, malt, and often hop. 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 α-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. Unfortunately, the isohomulones have pronounced light sensitivity. After light exposure, they develop a repulsive taste and skunky odor due to reactions with the sulfur-containing 3-methyl-2-buten-1-thiol (skunk thiol)[2]. This phenomenon is termed lightstruck flavor.

To prevent the development of lightstruck flavor, reduced isohumulones such as tetrahydro-isohumulones (tetrahydro-iso-α-acids) are often used in the brewing process to enhance both light and foam stability of beer[3]. In Germany, the addition of artificially reduced isohumulones is prohibited due to the Reinheitsgebot (purity requirements)[4], which states that only natural hop compounds are allowed in the brewing process. Because of their key role in the flavor characteristics and of the stringent quality control, it is very important to accurately determine and quantify isohumulones in beer.



Humulone *cis*-Isohumulone *trans*-Isohumulone

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

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-α-acid per L. Bitterness is traditionally analyzed using spectrometric analyses[5]. 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].

This Application Note shows the analysis of isohumulones and reduced isohumulones using the Agilent 1220 Infinity Mobile LC Solution as a robust and rugged system, resistant against shocks or vibrations during transportation. For the analysis of isohumulones in beer, no time-consuming sample preparation like solid phase extraction (SPE) or complex method development is necessary. Due to direct injection of the beer samples and isocratic elution, also less experienced HPLC users like brewers, for example, are able to measure their beer samples. With this simple setup, it is possible to perform easy on-site measurement of beer in a mobile laboratory.

Experimental

The Agilent 1220 Infinity Gradient LC system with DAD (G4294B) was equipped with a dual gradient pump with integrated degasser, autosampler, column compartment, and the diode array detector. For transportation, the LC can be mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

Software

OpenLAB CDS ChemStation Edition for LC/LC/MS Systems, Rev. C.01.05 [35]

Solvents and samples

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Ethylenediaminetetraacetic acid (EDTA) was purchased from Fluka (Sigma-Aldrich), St. Louis, USA. Phosphoric acid was purchased from Merck, Darmstadt, Germany.

DCHA-Iso, ICS-I3 (purified preparation of the dicyclohexylamine salts of *trans*-iso-α-acids) and Tetra ICS-T2 (purified preparation of tetrahydroiso-α-acids containing both *cis* and *trans* isomers) were purchased from Labor Veritas AG, Zurich, Switzerland. Different types of beer were bought in local stores. The beer samples were degassed by extensive stirring (10 minutes) with subsequent sonication (10 minutes) before injection to the HPLC system.

Chromatographic conditions

The analysis was carried out using an Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column (p/n 695975-902). Table 1 shows the chromatographic conditions.

Results and Discussion

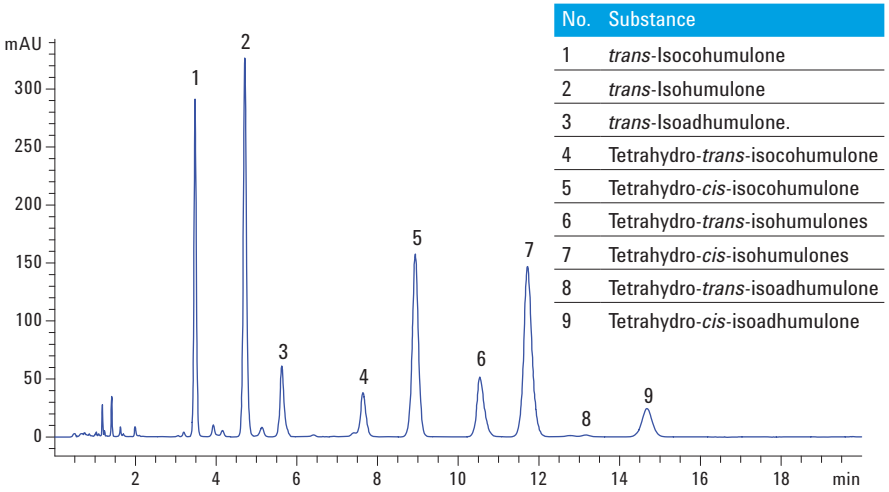
Isohumulone standards were separated isocratically using the mobile phase described in Table 1. All nine peaks were well separated (Figure 2). The addition of EDTA ensured optimal peak shape and, therefore, improved resolution in comparison to eluents without EDTA (data not shown). Three nonreduced *trans*-isohumulone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

The analysis was very precise for six consecutive runs with relative standard deviation (RSD) for retention time below 0.11 %. The RSD for the area was below 0.2 % except for Peak 8.

In addition, the linearity was evaluated with a standard curve using eight different concentration levels (from 3.33 mg/mL to 1.5 µg/mL, 1:3 dilution). The linear relationship was determined between the peak area and the corresponding concentrations. The method showed high linearity with correlation coefficients over 0.999 for all nine isohumulone standards. Table 2 displays the correlation coefficients together with the RSD values for retention time (RT) and area.

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.

Table 2. Linearity and precision in RSD (%) for all nine analyzed iso-α-acids.

No.	Iso-α-acids	Correlation coefficient	RT RSD (%)	Area RSD (%)
1	*trans*-Isocohumulone	0.99987	0.097	0.199
2	*trans*-Isohumulone	0.99987	0.083	0.157
3	*trans*-Isoadhumulone	0.99988	0.099	0.158
4	Tetrahydro-*trans*-isocohumulone	0.99989	0.102	0.093
5	Tetrahydro-*cis*-isocohumulone	0.99988	0.065	0.148
6	Tetrahydro-*trans*-isohumulones	0.99989	0.092	0.142
7	Tetrahydro-*cis*-isohumulones	0.99987	0.062	0.148
8	Tetrahydro-*trans*-isoadhumulone	0.99957	0.089	0.693
9	Tetrahydro-*cis*-isoadhumulone	0.99989	0.064	0.145

To determine and quantify the isohumulones in real beer samples, 14 beer samples were analyzed. The IBUs were determined in four top-fermented and 10 bottom-fermented beer samples, where 1 IBU equals 1 mg of dissolved iso-α-acid per liter. The amount of the isohumulones in the samples was calculated using the standard curve. As expected, there was a significant difference in the isohumulone content between the various types of beer. Mild nonbitter beers such as weizen or lager beer contained less isohumulone and had a smaller IBU compared to the more bitter beers, such as Irish stout or pils. The experimental values for the IBUs were compared to the IBUs found in the literature (Beer Judge Certification Program, Inc.). The determined IBUs were all within the literature given IBU range. Table 3 summarizes the results from the experimental IBU determination and the literature values for all 14 analyzed beer samples.

After extensive stirring of the beer samples, they were directly injected into the 1220 Infinity LC System without solid phase extraction (SPE) or further sample preparation. Figure 3 shows the analysis of (A) German Kölsch beer containing all *cis/trans* isomers of isocomumulone, isohumulone, and isoadhumulone. In addition to the variances in peak intensity, all the other beer samples revealed almost the same peak pattern of the nonreduced isohumulones except for the American premium lager. Only the American premium lager, as shown in Figure 3B, contained reduced isohumulones (tetrahydro-isocohumulones, tetrahydro-isohumulones, and tetrahydro-isoadhumulones) instead of the nonreduced ones.

Table 3. Comparison of IBUs found in literature to experimental data. *The literature IBUs were taken from the Beer Judge Certification Program, Inc.

Yeast type	Beer type	IBU experimental	IBU literature*
top-fermented *(Saccharomyces cerevisiae)*	Weizen	11	8–15
	Kölsch	23	20–30
	Irish stout	40	30–45
	Northern English brown ale	24	20–30
bottom-fermented *(Saccharomyces carlsbergensis)*	Premium lager	26	18–30
	American premium lager	19	8–15
	Lager	14	8–15
	Export	27	23–30
	Bock	29	23–35
	Pils	26	25–45, partly up to 100
	Pils	38	
	Pils	27	
	Pils	60	
	Pils, alcohol-free	49	

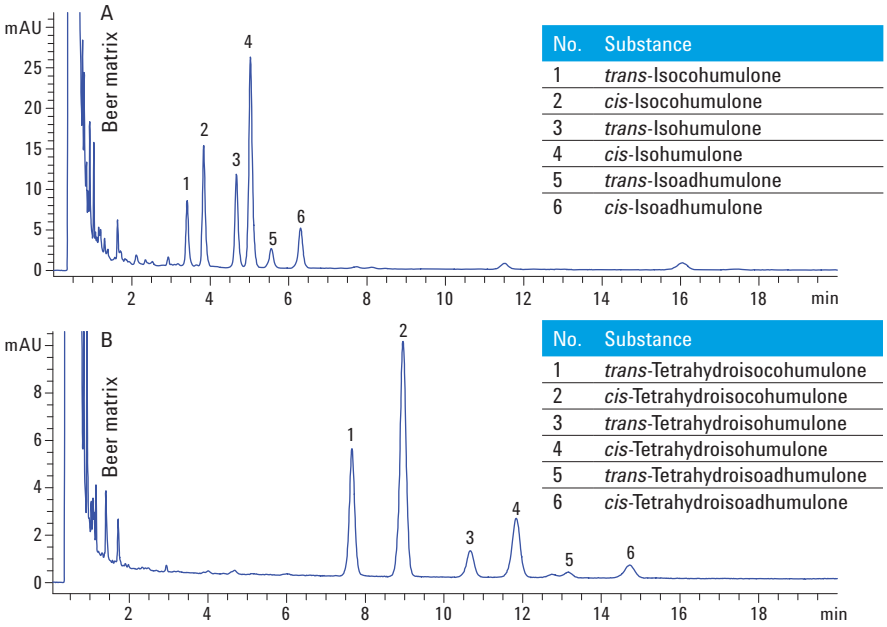


Figure 3. Separation of (A) isohumulones in German Kölsch and of (B) reduced isohumulones in American premium lager. All *cis/trans* isoforms from nonreduced (A) and reduced (B) isocohumulone, isohumulone and isoadhumulone were well separated.

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.

References

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