

Introduction

This Application Note shows the analysis of isohumulones and reduced isohumulones (trans-tetrahydro-iso- α -acids) in different types of beer. Beer is an alcoholic beverage produced by fermentation of the basic ingredients water, malt and often hop. 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.

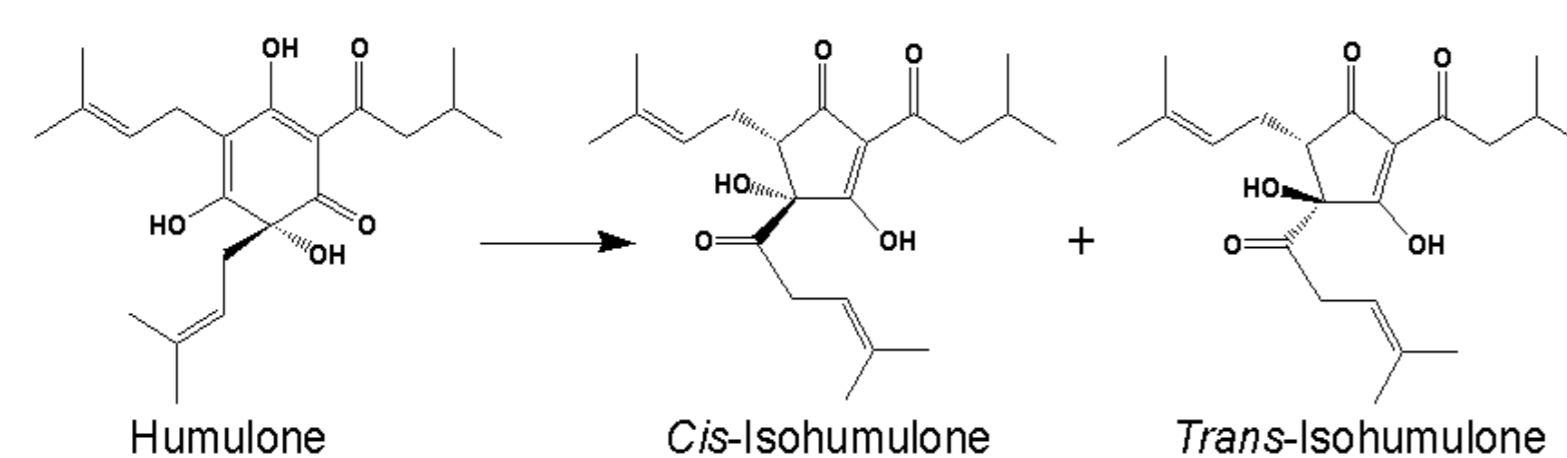


Figure 1 – Thermal isomerization of humulones to isohumulones during the brewing process.



Agilent 1220 Infinity Mobile LC Solution

Isohumulones contribute highly to the typical beer flavor like the bitter taste, but they have also bacteriostatic properties and an important function for foam stability. Unfortunately, the isohumulones have pronounced light sensitivity. After light exposure, they develop so called "lightstruck flavor" due to reactions with the sulfur-containing worth leading to 3-methyl-2-buten-1-thiol (skunk thiol).

To prevent the development of "lightstruck flavor", reduced isohumulones like tetrahydro-isohumulones are often used in the brewing process. In Germany, the addition of non-natural reduced isohumulones is prohibited due to the "Reinheitsgebot" (purity requirements), 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 of great interest to accurately determine and quantify isohumulones in beer. The bitterness in beers is measured in IBU (International Bitterness Units).

Here, we show 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.

Due to direct injection of the beer samples and isocratic elution, also unexperienced 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

Systems

Agilent Infinity 1220 LC System with Diode Array Detector with 1220 Infinity Mobile Upgrade Kit.

Software

OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
OpenLAB CDS 3D UV Add-On software.

Solvents & Samples

All solvents were LC grade. DCHA-Iso, ICS-I3 and Tetra ICS-T2 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 min) with subsequent sonication (10 min) before injection to the HPLC system.

Chromatographic conditions

The analysis was carried out using Agilent Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 μ m column.

Table 1 - Chromatographic conditions	
Solvent	ACN/H ₂ O + H ₃ PO ₄ to pH 2.8 (52:48, v/v) + 1mL EDTA 0.1M per liter solvent
Flow rate	1.8 mL/min
Stoptime	20 min
Injection volume	5 μ l (standards) or 20 μ l (beer samples) injection with needle wash
Temperature TCC	35°C
Wavelength	270 nm/ 4 nm, Ref.: OFF
Peak width	>0.025 min (0.5 s response time) (10 Hz)

Results and Discussion

All nine peaks of the isohumulone standards were well separated, see Figure 2. Three non-reduced trans-isohumulone and six reduced isohumulone standards were used for the evaluation of precision and linearity.

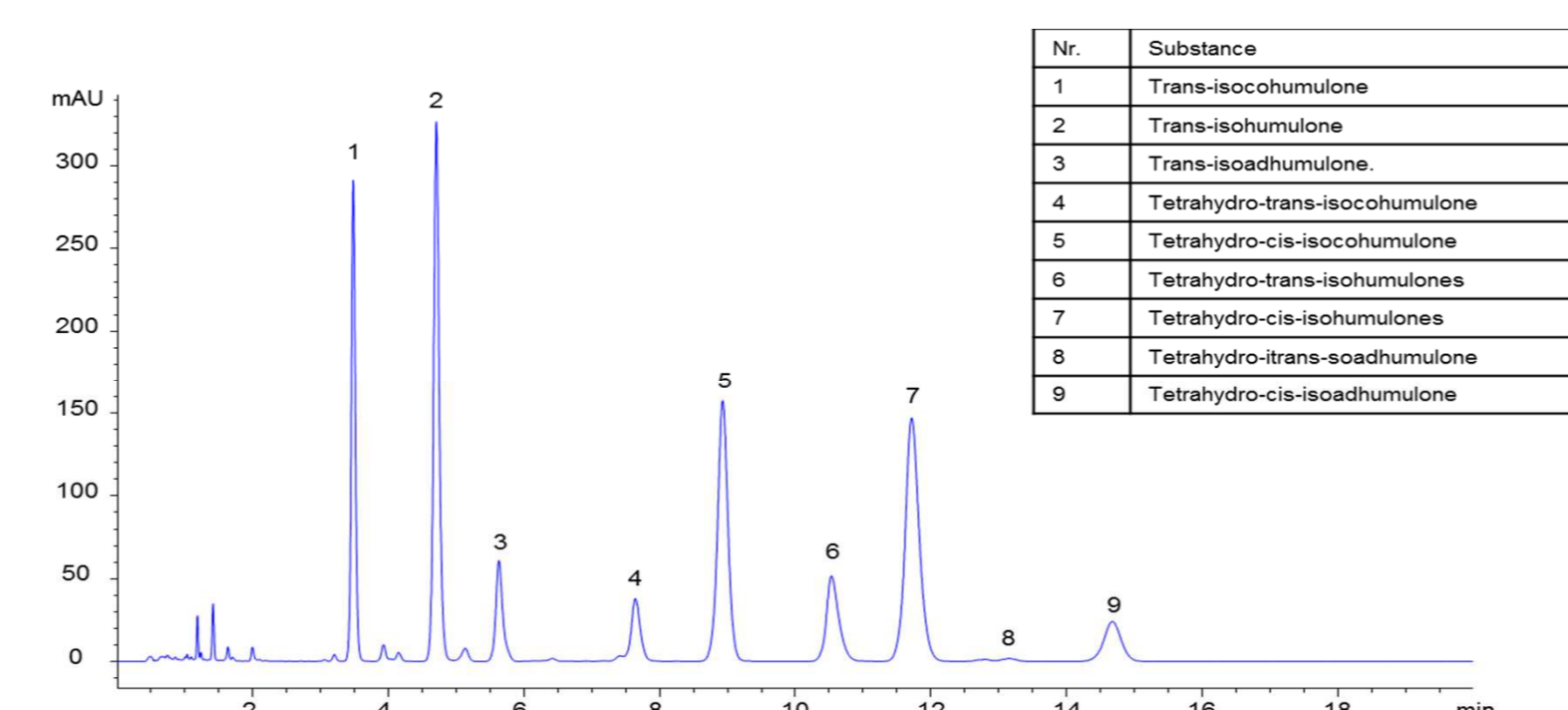


Figure 2 – Separation of nine isohumulone standards, non-reduced (Peak 1-3) and reduced (Peak 4-9) with isocratic elution.

The analysis was very precise and linear with correlation coefficients over 0.999 for all nine isohumulone standards. The international bitterness units (IBU) were determined in four top-fermented and ten bottom-fermented beer samples, where 1 IBU equals 1 mg dissolved iso- α -acid per liter. The amount of the isohumulones in mg was calculated using the standard curve.

Like expected, there was a significant difference in the isohumulone content in the various types of beer, see Table 2.

After extensive stirring of the beer samples, they could be directly injected into the 1220 Infinity LC System without further sample preparation. Figure 3 shows the analysis of (A) German Kölsch. Only the American Premium Lager, as shown in Figure 3B, contained reduced isohumulones (tetrahydro-isochumulones, tetrahydro-isohumulones and tetrahydro-isoadhumulones) instead of the non-reduced ones.

Table 2 - Comparison of International Bitter Units (IBU) 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 (<i>Saccharomyces cerevisiae</i>)	Weizen	11	8-15
	Kölsch	23	20-30
	Irish Stout	40	30-45
	Northern English Brown Ale	24	20-30
bottom-fermented (<i>Saccharomyces carlsbergensis</i>)	Premium Lager	26	
	American Premium Lager	19	18-30
	Lager	14	8-15
	Export	27	23-30
	Bock	29	23-35
	Pils	26	
	Pils	38	
	Pils	27	25-45, partly up to 100
Pils, alcohol-free	60		
	Pils, alcohol-free	49	

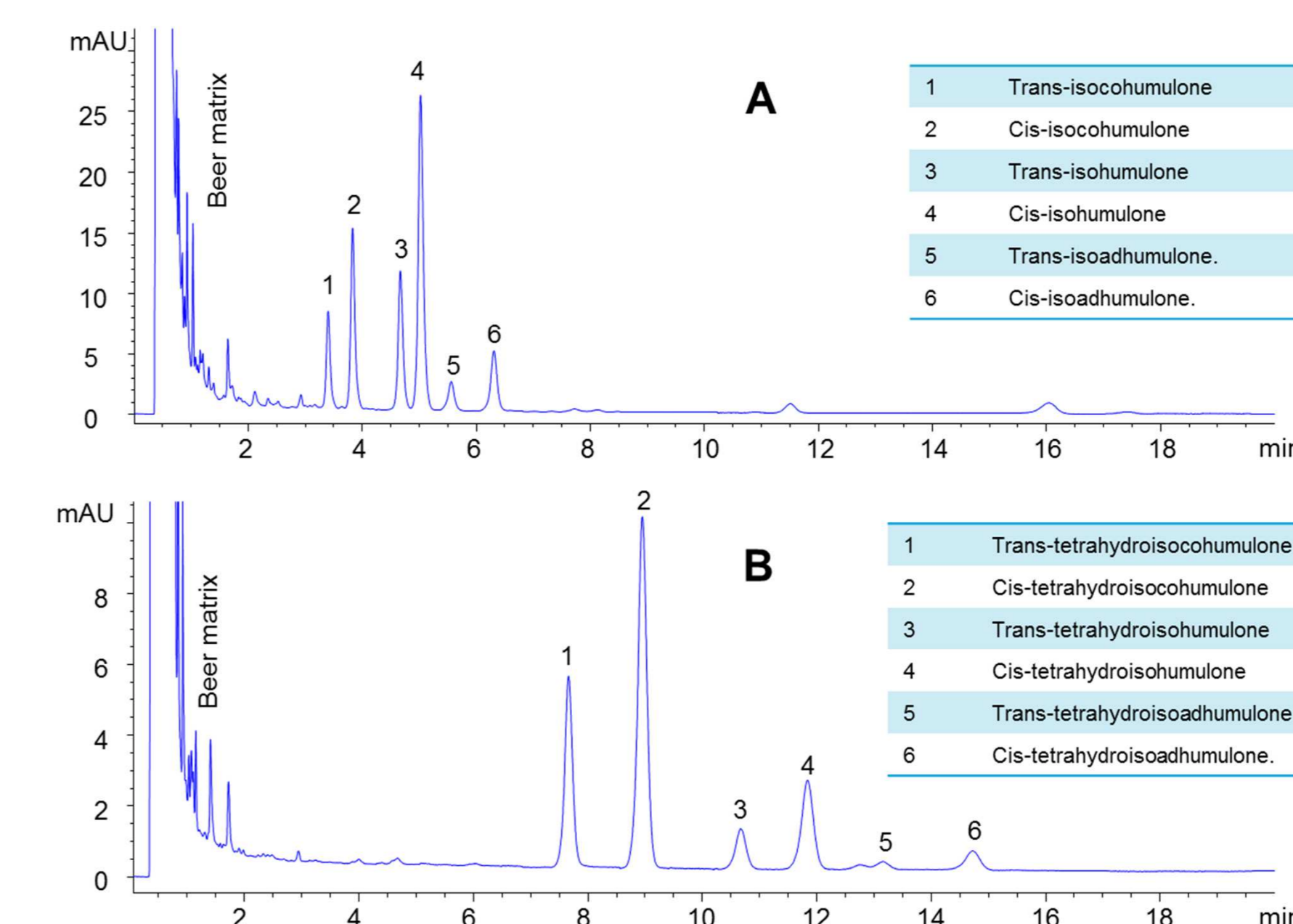


Figure 3 – Separation of (A) isohumulones in German Kölsch and of (B) reduced isohumulones in American Premium Lager.

Conclusions

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 also unexperienced users to perform isohumulone analysis in beer. The Agilent 1220 Infinity Mobile LC Solution, as a robust and rugged system enables easy on-site measurement of isohumulones in beer in a simple analytical setup.

References:

1. Statistisches Bundesamt, Germany.
2. Burns et al., Mechanism for Formation of the Lightstruck Flavor in Beer Revealed by Time-Resolved Electron Paramagnetic Resonance. *Chem. Eur. J.*, 7 (21): 4553-4561, 2001.
3. Vanhoenacker et al., Analysis of iso- α -acids and reduced iso- α -acids in beer by direct injection and liquid chromatography with ultraviolet absorbance detection or with mass spectrometry. *Journal of Chromatography A*, 1035: 53-61, 2004.
4. Vorläufiges Biergesetz § 9.