Fingerprinting Analysis of Different Types of Beer Using Comprehensive 2D-LC

Agilent 1290 Infinity 2D-LC Solution

Application Note

Food Testing & Agriculture

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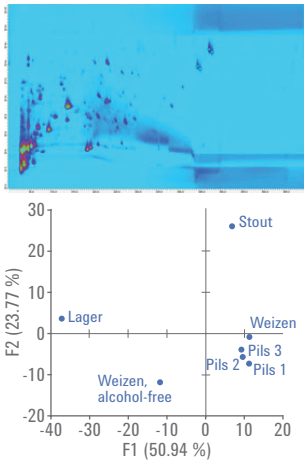
Abstract

Beer is an alcoholic beverage of highly complex composition and is produced from the basic ingredients water, malted barley or wheat, and for most beers hops. The typical bitterness of beer originates mainly from iso-α-acids that are formed from α-acids contained in hops during wort boiling. This Application Note demonstrates the comprehensive 2D-LC analysis of different types of beer using the Agilent 1290 Infinity 2D-LC solution. Good orthogonality was achieved using C18 columns at alkaline and acidic pH values in the first and second dimension respectively. Identification of beer bitter compounds was performed based on comparative analysis of standard substances and MS detection. Nontargeted, multisample analysis (fingerprinting analysis) enabled a classification of the different types of beer analyzed.



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Introduction

Beer is an alcoholic beverage produced by saccharification of starch and fermentation of the resulting sugar. The basic ingredients of beer are water, malted barley or wheat, and for most beers hops[1,2]. Stages of beer processing include malting, brewing, fermentation, and maturation. With more than 800 organic compounds contained in beer, its composition is highly complex. The distinctive tastes of different beers mainly originate from the mineral content of the water and the types of ingredients used as well as differences in the brewing methods. Regarding fermentation, there are two classical beer styles; top-fermented such as ale and German weizen beer, and bottom-fermented such as lager and German pils beer[1].

The typical beer bitterness is achieved by adding hops (*Humulus lupulus* L.) cones, pellets, or extracts during wort boiling. Hops contain α-acids (humulones) and β-acids (lupulones), which are mainly present in form of their *n*-, co- and ad-homologs. During wort boiling, the hop α-acids, which are almost tasteless, are transformed into iso-α-acids (isohumulones) that are responsible for the typical beer bitterness and the stability of beer foam. Iso-α-acids are light sensitive, and light exposure leads to the formation of off-flavors (light struck flavor)[3-5]. For this reason, reduced iso-α-acids, such as tetrahydroiso-α-acids, are used in the brewing industry to enhance the light and foam stability of beer. In Germany, the addition of reduced iso-α-acids is prohibited by the *Reinheitsgebot*, which mandates that only natural hop compounds may be used[4].

The analysis of α-acids and β-acids in hop products as well as of iso-α-acids and reduced iso-α-acids in beer can be accomplished by high performance liquid chromatography (HPLC) with ultraviolet (UV) or mass spectrometric (MS) detection[3-5]. The quantification of iso-α-acids and reduced iso-α-acids in beer was shown in a previous Application Note[6].

In addition to α-acids and β-acids, hops also contain many polyphenolic compounds, some of which add to the typical beer bitter taste. The most important polyphenolic compounds are xanthohumol-related prenylflavonoids, such as xanthohumol, isoxanthohumol, and desmethylxanthohumol[3,5].

Figure 1 shows the structures of iso-α-acids and reduced iso-α-acids as well as of xanthohumol-related prenylflavonoids.

Because of the highly complex composition of beer, comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC) with its inherent high peak capacity is ideally suited for a comprehensive analysis of beer. This Application Note shows the fingerprinting analysis of different types of beer which enables a classification of the analyzed beer samples.

Experimental

Equipment

The Agilent 1290 Infinity 2D-LC solution was comprised of the following modules:

- Two Agilent 1290 Infinity Binary Pumps (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A) with an Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/4-port duo valve (2D-LC valve head, 1,200 bar (p/n 5067-4214) equipped with two 60-µL loops
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light 60-mm cartridge cell (G4212-60007)

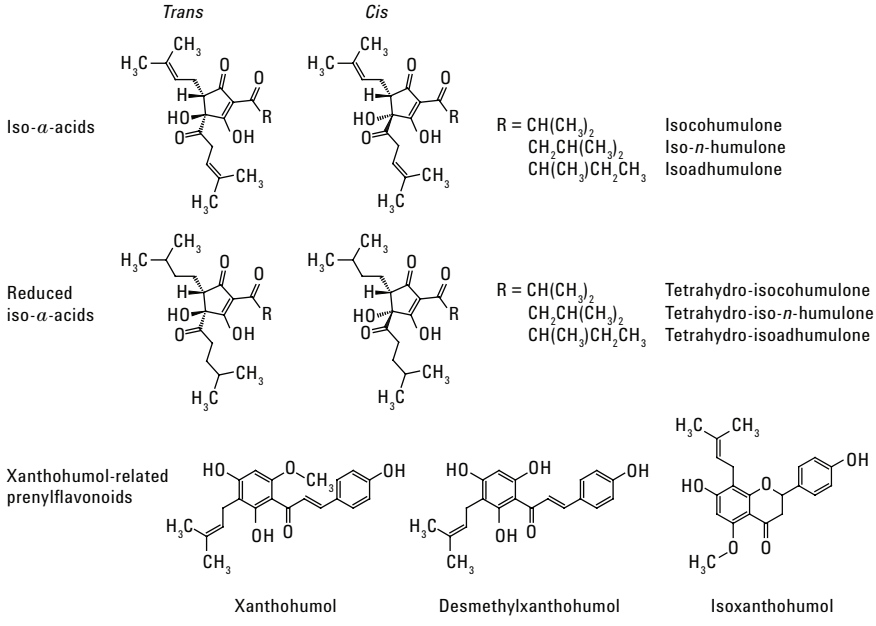

Figure 1. Structures of iso-α-acids, reduced iso-α-acids, and xanthohumol-related prenylflavonoids.

Mass spectrometric detection was performed using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system equipped with an Agilent Jet Stream ESI source (G1958-65538).

Software

- Agilent OpenLAB CDS ChemStation Edition rev. C.01.06 [61] with Agilent 1290 Infinity 2D-LC Acquisition Software product version A.01.01 [26]
- Agilent MassHunter Workstation Software, LC/MS data acquisition for Agilent 6200 series TOF/6500 series Q-TOF version B.05.01, qualitative analysis version B.06.00
- GC Image LCxLC-HRMS Edition software for 2D-LC data analysis from GC Image LLC., Lincoln, NE, USA

Columns

First dimension

Agilent ZORBAX Extend-C18 Narrow-Bore RR, 2.1 × 100 mm, 3.5 µm (p/n 761753-902)

Second dimension

Agilent Poroshell HPH-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-702)

Chemicals

All solvents were LC grade. Acetonitrile and ethanol were purchased from Merck, Darmstadt, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). 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. Ammoniumacetate was obtained from Sigma-Aldrich, Steinheim, Germany and Ammonia Solution was purchased from Merck, Darmstadt, Germany. Formic acid was from Agilent (p/n G2453-85060).

Samples and sample preparation

Different types of beer were bought in local stores (Germany). The beer samples were degassed by stirring (10 minutes) and sonication (10 minutes). Before injection into the HPLC system, the samples were filtered using a 1-mL plastic syringe with Captiva Premium Syringe Filters Regenerated Cellulose, 15 mm, 0.45 µm (p/n 5190-5109).

Comprehensive 2D-LC method

First dimension pump	
Solvent A	5 mM Ammonium acetate in water, adjusted to pH 9.95 with ammonia
Solvent B	Acetonitrile/Ethanol (60/40; v/v)
Flow rate	0.075 mL/min
Gradient	0 minutes – 2 %B 10 minutes – 2 %B 40 minutes – 40 %B 60 minutes – 50 %B 61 minutes – 95 %B 70 minutes – 95 %B
Stop time	70 minutes
Post time	15 minutes
Second dimension pump	
Solvent A	Water + 0.25 % formic acid
Solvent B	Acetonitrile + 0.25 % formic acid
Flow rate	4.0 mL/min
Gradient and gradient modulation	0.00 minutes 5 %B; 20 minutes 5 %B; 43 minutes 30 %B; 44 minutes 70 %B; 49 minutes 70 %B; 50 minutes 55 %B 0.24 minutes 40 %B; 20 minutes 40 %B; 43 minutes 85 %B; 44 minutes 92 %B 0.25 minutes 5 %B; 20 minutes 5 %B; 43 minutes 30 %B; 44 minutes 70 %B; 49 minutes 70 %B; 50 minutes 55 %B 0.35 minutes 5 %B; 20 minutes 5 %B; 43 minutes 30 %B; 44 minutes 70 %B; 49 minutes 70 %B; 50 minutes 55 %B

The first and second dimension gradients are displayed in Figure 2.

Thermostatted column compartment

- First dimension column on the right side at 25 °C
- Second dimension column on the left side at 30 °C

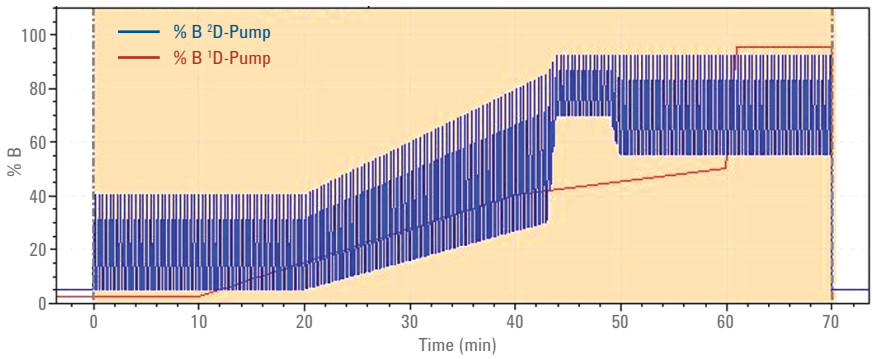

Figure 2. First and second dimension gradients.

2-position/4-port duo valve

The 2-position/4-port duo valve was switched automatically after each second dimension modulation cycle of 21 seconds. The loops were used in a cocurrent manner (filling and elution of the loops in the same flow direction).

Autosampler

Injection volume	10 µL
Sample temperature	6 °C
Needle wash	6 seconds in methanol

Diode array detector

Before detection, the effluent from the second dimension column was split approximately 7:1 between the DAD and the MS using a T-piece. The connection from the T-piece to the MS was made using a 0.075-mm id capillary (340 mm length) to minimize peak broadening.

Wavelength	270 nm/4 nm, Ref. 395 nm/10 nm
Data rate	80 Hz

Mass spectrometer

The 6530 Accurate-Mass Q-TOF LC/MS system was operated in negative ionization mode with an acquisition rate of 10 spectra/second, and the following Jet Stream ESI source conditions.

Gas temperature	300 °C
Gas flow	9 L/min
Nebulizer	50 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary	–3,000 V
Nozzle	–1,000 V

Results and Discussion

A comprehensive 2D-LC method was developed for the fingerprinting analysis of different types of beer. The first dimension separation used an Agilent ZORBAX Extend-C18 column at alkaline pH, as was previously described for the analysis of iso-α-acids and reduced iso-α-acids in beer[4]. In the second dimension, an Agilent Poroshell HPH-C18 column was used with acidic pH to obtain selectivity differences due to different pH values in the first and second dimension separation, and thereby enhance orthogonality. Because of the injection of alkaline first dimension effluent to the second dimension column with every modulation cycle, a column that is stable under alkaline conditions was used in the second dimension.

Using the developed comprehensive 2D-LC method, seven beer samples (three different German pils beers, one German weizen beer, one German alcohol-free weizen beer, one Irish stout beer and one American lager beer) were analyzed in triplicate. Figure 3 shows the chromatograms of the analyses of one German weizen beer, one German pils beer and one American lager beer with UV detection at 270 nm. It can be seen that a good coverage of the two-dimensional separation space was achieved by using C18 columns with alkaline and acidic pH values in the first and second dimension separations, respectively. Additionally, differences in the peak pattern (fingerprint) can be observed for the three different types of beer.

In Figure 3, the peaks corresponding to iso-α-acids and reduced iso-α-acids are marked. Identification was based on comparative analysis of standards (a mixture of *trans*-iso-α-acids as

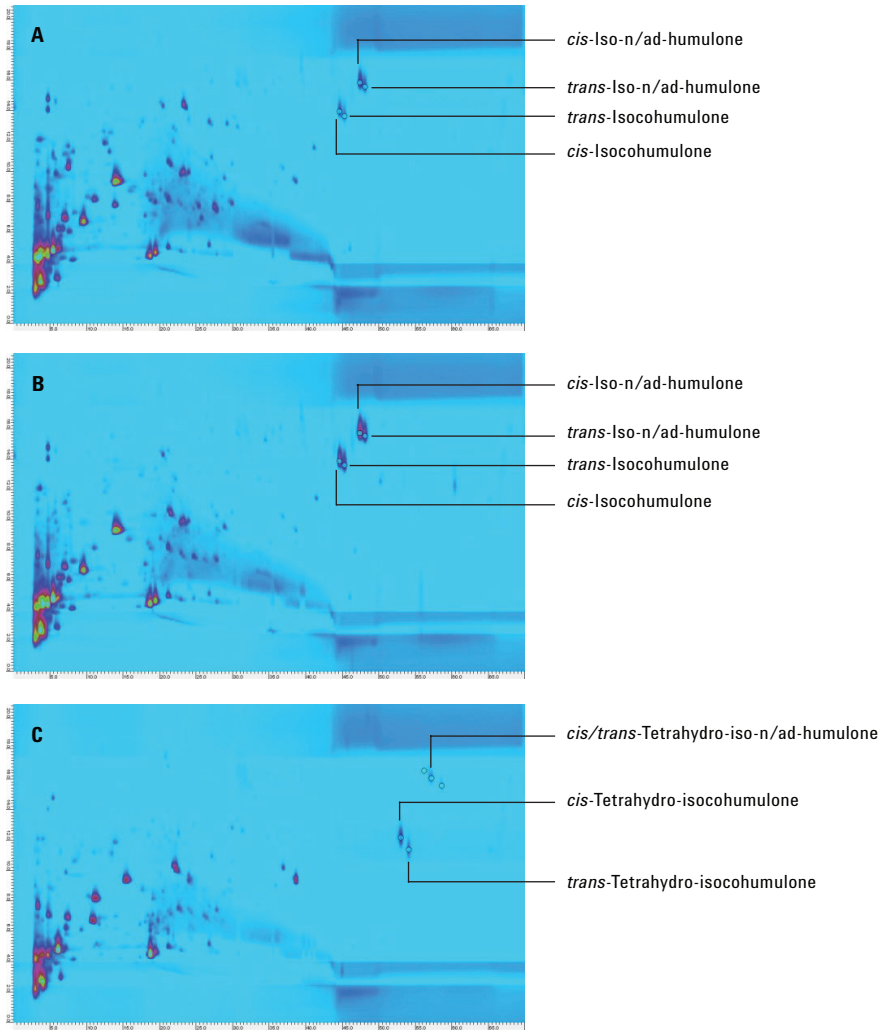

Figure 3. Chromatograms from the analyses of different beer samples with UV detection at 270 nm; A) German weizen beer; B) German pils beer; C) American lager beer.

well as a mixture of reduced *cis*- and *trans*-iso-α-acids was analyzed) and on MS detection. Figure 4 shows exemplarily the mass spectra of the *cis*-tetrahydro-isocohumulone peaks from the analysis of the standard mixture of reduced *cis*- and *trans*-iso-α-acids (A) and from the analysis of the American lager beer (B). The mass spectra are very comparable, and the $[M-H]^-$ peak showed mass differences of 0.9 ppm and 0.6 ppm for the analysis of the standard and the American lager beer, respectively.

The analyzed German beers and the Irish stout beer all contained iso-α-acids and no reduced iso-α-acids, as can be expected from beers brewed according to the German *Reinheitsgebot* (using only natural hop compounds). Conversely, the analyzed American lager beer contained reduced iso-α-acids, and no iso-α-acids were detected.

For comparison and classification of different samples, a nontargeted, multisample analysis comparing every constituent in every sample can be performed using the LCxLC software as was described in a previous Application Note[7]. For this nontargeted, multisample analysis, a good reproducibility of retention times and peak volumes is needed, which is offered by the Agilent 1290 Infinity 2D-LC Solution[8].

For comparison and classification of the different types of beer analyzed, the chromatograms obtained from the comprehensive 2D-LC analysis with UV detection at 270 nm were used. After preprocessing each chromatogram with baseline correction and peak detection using the LCxLC software, the Image Investigator software (part of the LCxLC software) was used to perform a cross sample feature matching. During this process, all chromatograms were aligned and a composite chromatogram was generated. The composite chromatogram contains all peaks from all chromatograms and is used to define peak-region features (feature areas). For each chromatogram, the percent response for each of the defined feature areas is calculated. The feature areas with their respective percent responses can be used for comparison and classification of the different types of beer analyzed.

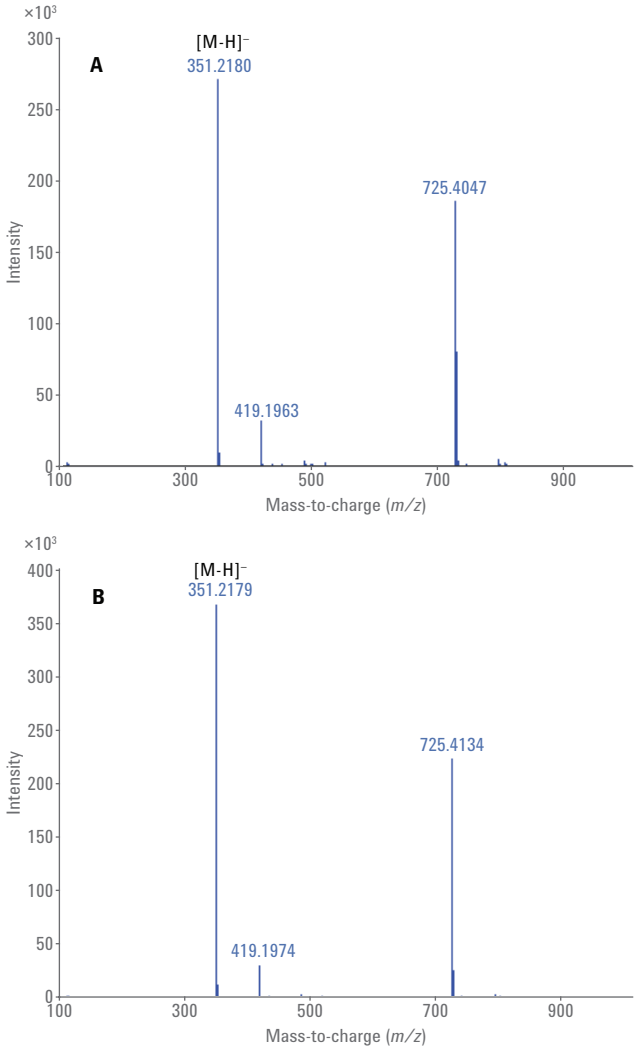

Figure 4. Mass spectra of the *cis*-tetrahydro-isocohumulone peaks from the analysis of the standard mixture of reduced *cis*- and *trans*-iso-α-acids (A) and from the analysis of the American lager beer (B).

Classification of the analyzed beer samples was performed by principal component analysis (PCA). For this purpose, an average percent response of each feature area was calculated for every beer sample from the three replicates analyzed. Using PCA, the beer samples could be classified according to their type, as shown in Figure 5. Approximately 88 % of the variance of the data is described by the first three principal components (F1-F3). Using F1 and F2, the American lager beer, the Irish stout beer, and the alcohol-free weizen beer are clearly separated from each other and from a group that consists of the three pils beers and the weizen beer analyzed. F3 further separates the weizen beer from the pils beers, which are closely grouped.

Conclusion

This Application Note demonstrates the comprehensive 2D-LC analysis of different types of beer using the Agilent 1290 Infinity 2D-LC solution. Good orthogonality was achieved using C18 columns at alkaline and acidic pH values in the first and second dimension, respectively. Identification of bitter beer compounds (iso-α-acids and reduced iso-α-acids) was performed based on comparative analysis of standards and MS detection. By nontargeted, multisample analysis using the LCxLC software followed by principal component analysis, it was possible to classify the different types of beer analyzed.

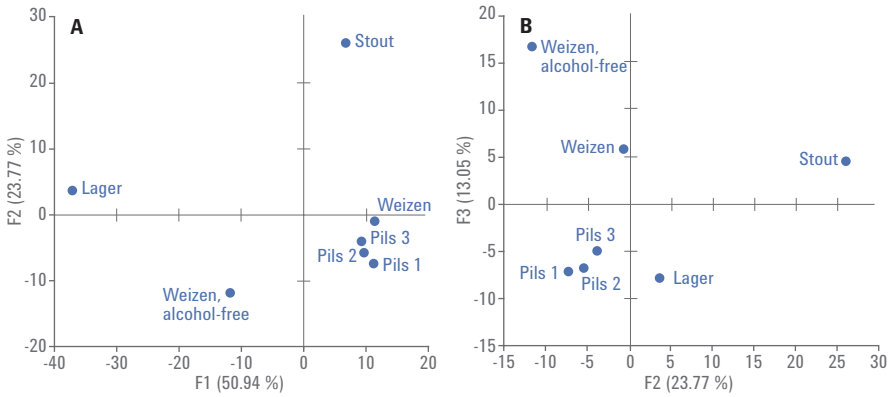

Figure 5. Principal component analysis of seven different beer samples. The first three principal components (F1–F3) describe approximately 88 % of the variance in the data.

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