500 Years German Beer Purity Law – Searching Contaminants

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■ Overview

Beer is the most popular alcoholic drink in Europe, and next to drinking water, it is one of the most researched food products with the highest standards regarding quality, freshness, appearance and flavor.

These standards have to do with the German Beer Purity Law of 1516 (the “Reinheitsgebot”), which uniquely defines the ingredients of beer to be hops, malt, yeast and water.

Figure1: Beer – the most popular alcoholic drink in Europe

Today, consumer interest in the diversity and variety of beers available has never been higher - of the 6,500 breweries in Europe, 700 have been established in 2014. Statistically, per capita beer consumption in European countries is around 68 liters a year. The highest consumption rate is in the Czech Republic (144 L) followed by Germany (107 L) and Austria (106 L). The popularity of beer and its high per capita consumption leads to the questions: how healthy is beer, and what ingredients does it contain?

■ European regulations

The quality standards for beer analysis are described in the European Brewery Convention (EBC), which includes the determination of elements like copper, zinc, sodium, potassium, calcium and more, anions such as nitrate and sulfite as well as organic components such as ethanol, glycerine and others. A meticulous quality control procedure is essential, and during each stage of the production process analytical methods such as spectroscopy, chromatography and mass spectrometry are applied for quality assurance or for product characterisation. For the quantitative determination of metals like copper and zinc, atomic absorption or ICP-OES spectrometry is the method of choice. Even though the most abundant constituent of beer is water, it is important to control all other constituents, which are dissolved in it. The determination of copper is important as high concentrations are disadvantageous on the colloidal stability and the taste of the beer. Same with zinc, which is an essential trace element for yeast influencing metabolic processes such as protein synthesis and nucleic acid metabolism. Typical concentration levels of copper and zinc in beer are 0.2 mg/L.

■ Quantitative Analysis of Heavy Metals

For quantitative determination of the elements in the required concentration range, ICP is the most preferable tool for quality control because of a high sensitivity, a wide dynamic range and a high sample throughput. The Shimadzu ICPE-9820 is a simultaneous spectrometer with CCD (charge-coupled device) detector, which has been used for all determinations. This instrument configuration is equipped with a unique optical system which sets new standards with respect to performance and speed and can be optimized for a wide variety of different applications.

The vacuum systems allows precise analysis of elements in the lower UV range under extremely stable conditions. The use of a vertical mounted mini torch allows a cooling gas flow rate of only 10 L/min. The system setup for determination of low concentration heavy metals in beer has been optimized using the mini torch in the dual view mode for axial and radial plasma observation, which allows the determination of high concentration elements such as alkaline and alkaline earth and the heavy metals at the same time. The beer samples have been simply diluted 1:1 with deionized water, and aspirated in the same way as aqueous solutions in the cyclone chamber and straight into the minitorch. The standard solutions have been prepared including an ethanol concentration of 2,5% in order to match the matrix. Table 2 shows a summary of the system parameters.

Parameter	Setting
RF generator power	1.2 kW
Cooling gas	10 l/min
Plasma gas	0.6 l/min
Carrier gas	0.7 l/min
Nebulizer	Coaxial
Plasma observation	Axial/Radial
Sensitivity	Wide Range
Exposure time	15 sec.
RF generator power	1.2 kW

Table 2: Analytical conditions for beer analysis

The typical concentration of copper in beer is expected at a maximum level of 0.2 mg/L. Higher copper concentrations are disadvantageous for the colloidal stability and the taste of the beer.

The calibration curve in Figure 2 shows the standards with concentrations starting at 250 µg/L up to the maximum concentration of 1000 µg/L. The limit of detection is calculated with <0,02 µg/L (3 s).

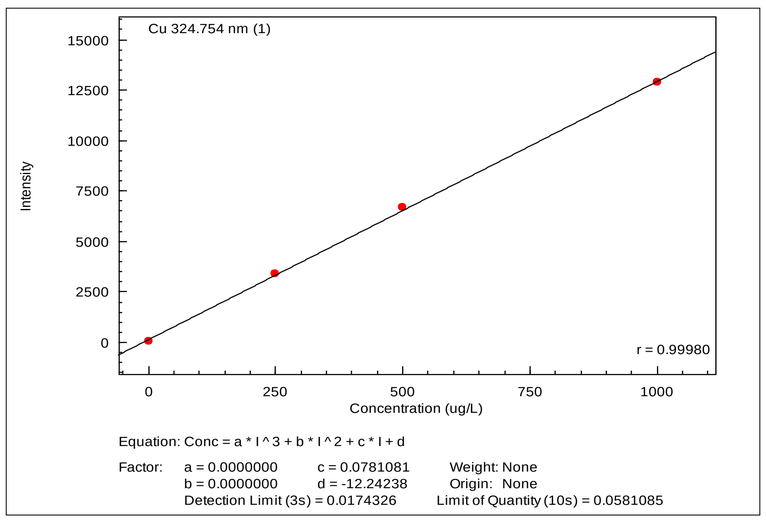

Figure 2: Calibration Curve of Copper

■ Pesticides in Beer?

Pesticides can be found as contaminants in brewing water and grains. In particular, the herbicide glyphosate, which has recently become the focus of the Munich Environmental Institute, has to be monitored carefully since it is probably carcinogenic. Elevated concentrations can be expected when glyphosate is applied to a crop shortly before the harvest, a process known as siccation. Siccation is already forbidden in Germany and Germany’s farm federation has reconfirmed that malt derived from glyphosate-sprayed barley has been banned; however, glyphosate could have been used on farms prior to the ban, meaning barley could still be grown in glyphosate-drenched soil.

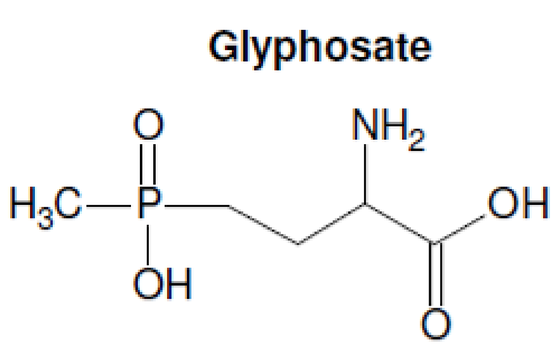

Figure 3: Glyphosate – the most popular pesticide for siccation

■ Determination of Glyphosate using LC-MSMS

The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well-established method including a derivatization step with 9-fluorenylmethyl chloroformate (FMOC) followed by LCMS analysis. However, this derivatization is time consuming and also susceptible to errors. Therefore, a sample pretreatment without derivatization is desirable as it is faster and cheaper.

The quantification of Glyphosate, based on derivatization to a method without any derivatization is compared here. The use of a high sensitivity mass spectrometer (LCMS-8060 coupled to a Nexera UHPLC, both from Shimadzu Corporation, Japan) allows to skip a sample concentration step by solid phase extraction (SPE) for both methods, which additionally reduces time and cost for the analysis.

1ml MeOH was added to 1 ml beer, vortexed and afterwards centrifuged for 15 min at 12000 rpm. 500 µL of the supernatant were used for the underivatised sample. For the derivatisation sample 25 µl EDTA-Borate buffer and 75 µl FMOC were added to another 500 µl of the supernatant. After 60 min incubation at 50 ° C we added 30 µl 0.2% phosphoric acid to stop the reaction. Finally, 125µl water were added.

Glyphosate could be quantified from 5 to 100 ng/mL with both methods. The calibration curves obtained for Glyphosate in beer are shown in Figure 4. Figure 5 shows representative chromatograms.

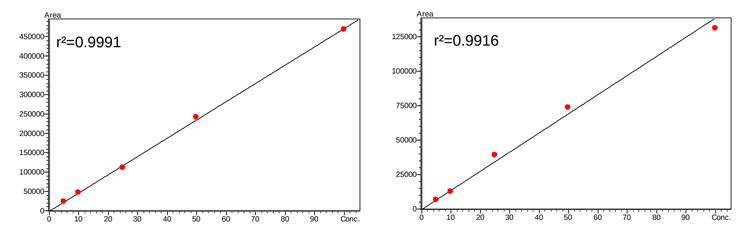

Figure 4: Calibration curves of Glyphosate determined in duplicate obtained from beer after sample pre-treatment. R² was better than 0.99.

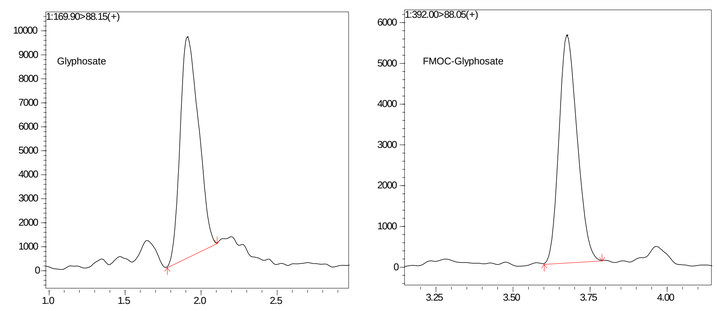

Figure 5: Exemplary chromatograms of a 15 ng/mL beer sample

Both methods allow the quantification of Glyphosat at 0.005 mg/kg (≙ 5 ng/mL) which is below the European Union maximum residue levels (MRL) for the raw material. The method without sample derivatization shows comparable sensitivity to the FMOC derivatization but sample pretreatment is much easier and quicker.

■ Summary

Beer is made from natural grain and vegetable base products which are exposed to environmental impacts as well as agricultural treatment. Beer may therefore contain a variety of heavy metals such as arsenic, lead and cadmium and additional undesired substances such as mycotoxins and pesticides. To track and analyze these elements and compounds various analytical technologies and sensitive analytical systems are required. Shimadzu is offering the full solution such as UV-Vis spectrophotometers, atomic absorption-, ICP-OES/MS spectrometers, liquid- and gas chromatography as well as mass spectrometry, and TOC analyzers to permanently guarantee the highest quality of the most popular alcoholic drink in Europe.

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