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# Determination of Organic and Inorganic Contaminants in Beer Using ICP-MS & LC-MSMS Spectrometry

Uwe Oppermann[1], Anja Grüning[1], Ludivine Fromentoux[2], and Jan Knoop[1]
[1]Shimadzu Europa GmbH, Spectroscopy, Duisburg, Germany, [2]Shimadzu France SAS, Marne La Vallee, France

## 1. 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. 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?

## 2. 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- and ICP-MS spectrometry is the method of choice.

Figure 1: Beer – the favourite drink in Europe

## 3. Multi Element Analysis

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. Furthermore, the determination of arsenic, antimony, cadmium, and lead is important, as those elements are toxic when they are present in beer or the brewing water. The source of these elements in beer and other alcoholic beverages could be the contamination of raw material and/or technological processes.

| Parameter | Setting |
|---|---|
| RF generator power | 1.2 kW |
| Plasma gas | 8 L/min |
| Auxiliary gas | 1.1 L/min |
| Carrier gas | 0.65 L/min |
| Nebulizer type | Micro-Mist |
| Sampling depth | 5 mm |
| Spray chamber temperature | 5 °C |
| Coll. cell gas flow (He) | 6 mL/min |
| Quantified isotopes | $Ni^{60}$, $Sb^{63}$, $Zn^{66}$, $As^{75}$, $Cd^{111}$, $Pb^{208}$ |
| Internal standards (ISTD) | $Ga^{71}$, $In^{115}$, $Tl^{205}$ |

Table 1: ICPMS 2030 measurement parameters

In this study the quantitative determination of the inorganic contaminants in beer, has been done using ICP-MS because of a high sensitivity (trace detection), a wide dynamic range and a high sample throughput. Shimadzu ICPMS-2030 is an easy and fast system to meet this requirement. Due to the unique Eco-mode system associated with Mini-torch, ICPMS- 2030 is able to drastically reduce running cost. Even though, beer is regarded as a difficult matrix because of the high number of constituents, the octopole collision cell assures a high accuracy for all element measurement. Using Helium gas and Kinetic Energy Discrimination principle, this cell suppressed most of the spectroscopic interferences (polyatomic interferences). Efficiency of interferences suppression and sensitivity are improved by a cooled cyclonic chamber and well controlled torch positioning.
For this study, two commercially available beers have been evaluated. The two beer samples analyzed here are undiluted and have been aspirated after degassing using the mesurement parameters listed in Table 1. An internal standard solution containing $^{71}Ga$, $^{115}In$, and $^{205}Tl$ was added using the automatic internal standard addition kit.

## 4. Experimental Conditions

The concentrations of As, Ni, Cd, Sb, Zn and Pb in the undiluted beer were determined using the calibration curve method. For each studied element, calibration curves are built using 4 standards in the concentration range from 2 to 10 µg/L. The standards have been prepared using a matrix-matched solution containing 5% ethanol. The calibration curves in Figure 2 shows all correlation coefficients are better than 0.999 and low levels of detection limits (LD) which are calculated automatically by LabSolution-ICPMS software with 3σ method. The data indicate that ICPMS-2030 is an ideal tool for trace contaminant analysis.

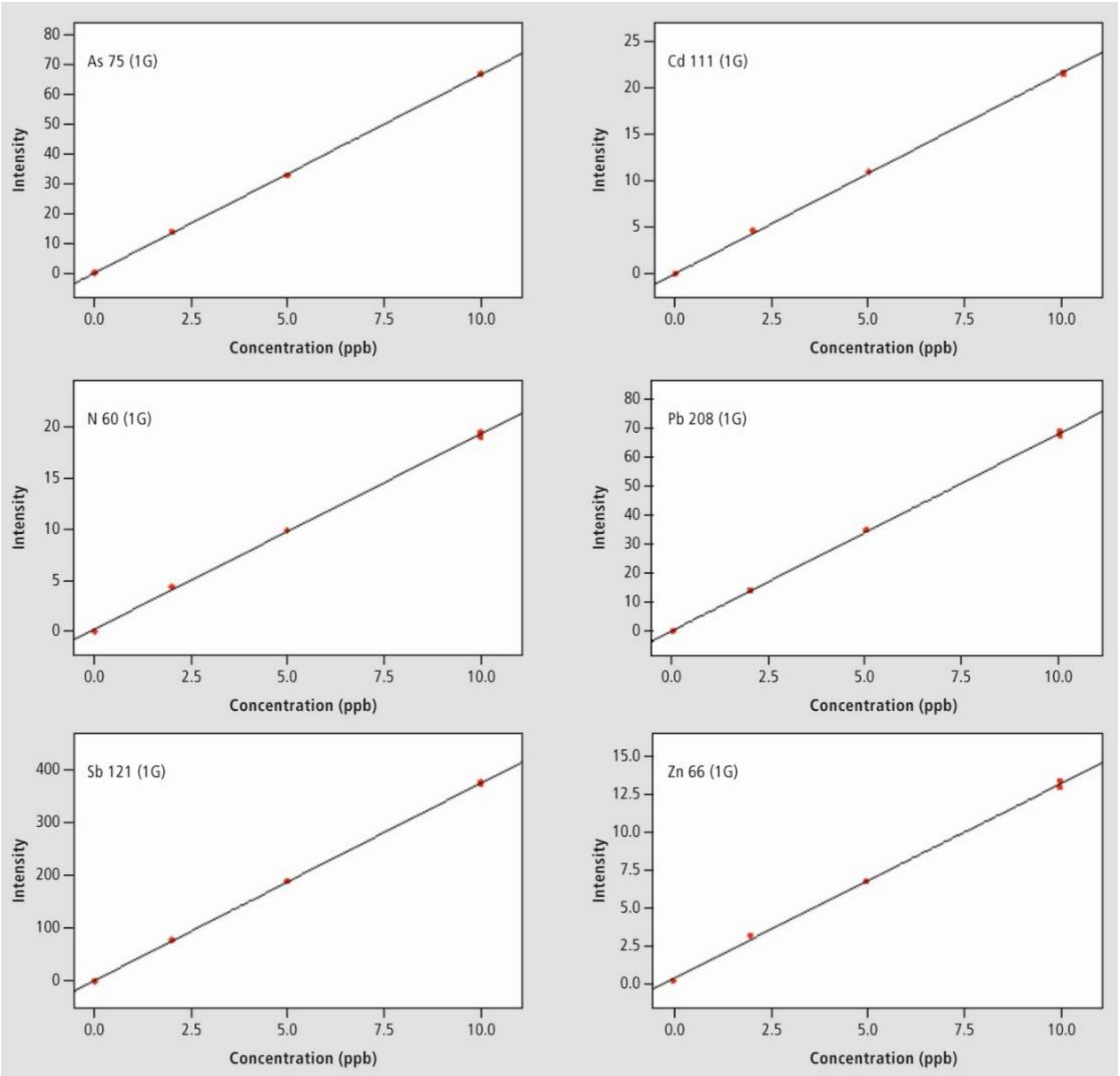


Figure 2: Calibration curves of $^{60}Ni$, $^{63}Sb$, $^{66}Zn$, $^{75}As$, $^{111}Cd$, $^{208}Pb$

## 5. 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 Europe and 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.

## 6. 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 3. Figure 4 shows representative chromatograms.

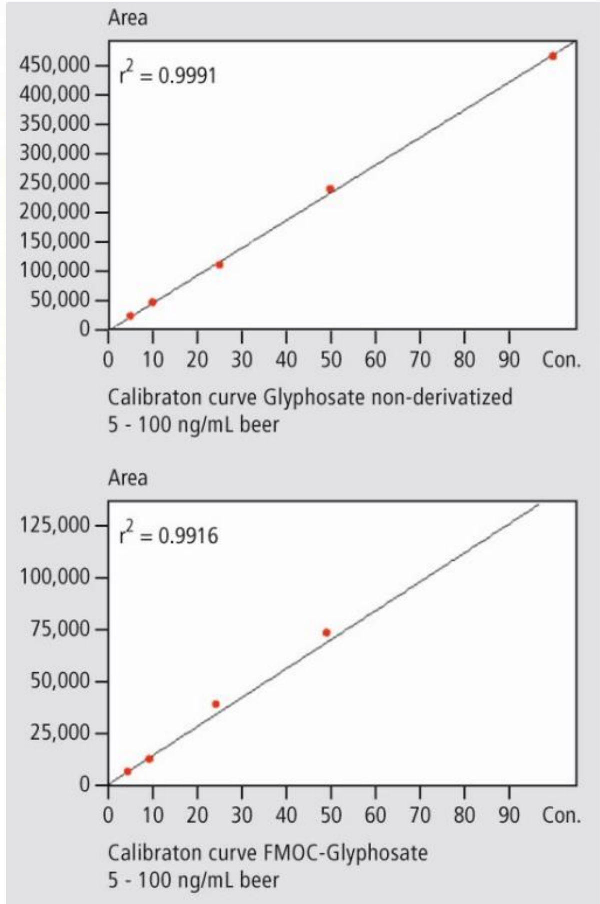


Figure 3: Calibration curves for Glyphosate in beer

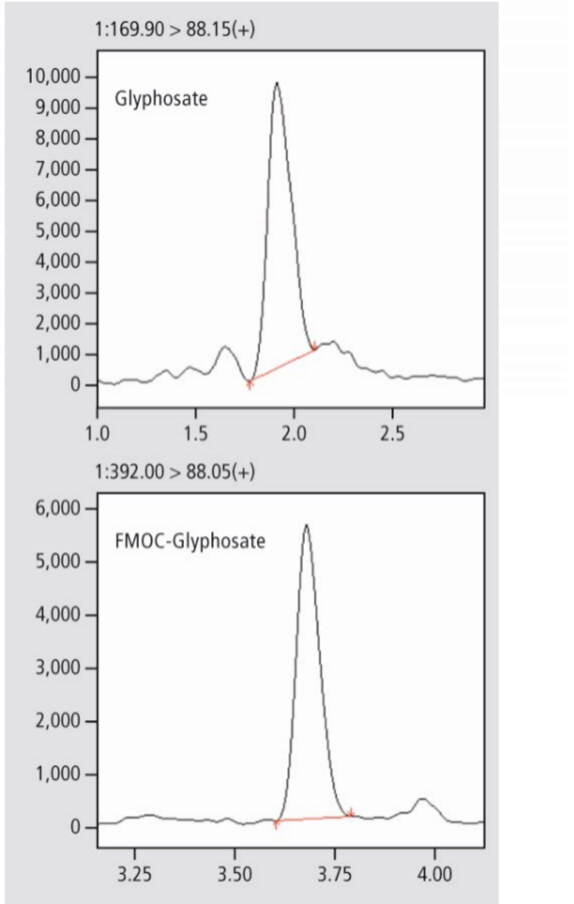


Figure 4: Chromatograms of a 15 ng/mL beer sample

## 7. Conclusion

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.