

Analytical and Testing Instruments for Beer

# Shimadzu's Total Support for Beer Analysis



# World Map of Shimadzu Sales, Service, Manufacturing, and R&D Facilities



# Shimadzu's Total Support for Beer Analysis

Shimadzu provides total support for beer analysis from farm to stein. As a leading manufacturer of a wide range of analytical instruments, Shimadzu undertakes development of new instruments and technology, and provides comprehensive service support in order to keep up with changing market demands.

In addition to offering analytical instruments and testing machines, Shimadzu provides total support that includes the provision of information for the beer community, training at workshops and seminars, and instrument maintenance management.

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# Introduction to Beer Analysis

In many philosophies, the classical four elements are earth, water, air, and fire. Analogous to this history is beer, which is made with four classical "elements": water, barley malt, yeast, and hops. Many varieties of beer are made using these ingredients, with barley malt or other grain sources are used in the brewing process. For flavoring, hops and other additives enhance the taste. Brewer's yeast, typically *Saccharomyces* (from the Greek as sugar fungus) ferments the sugars, extracted from the grain, into ethanol and carbon dioxide.

Brewing of beer dates back more than 5,000 years. There is even an ancient goddess of beer named Ninkasi. The Sumerians developed a hymn to her such that the recipe for beer could be passed down for generations. Today, "International Beer Day" is the first Friday in August and is celebrated in 207 cities in 50

countries on 6 continents.

Some of the larger organizations with specific testing for beer include the American Society of Brewing Chemists (ABSC) and the European Brewing Convention (EBC). The four "elements" in beer should be tested for characteristics, quality, and contamination to ensure the final product meets expectations. Beer brewing may appear simple with only four "elements" but the chemistry is quite complex, requiring many types of analytical instruments to obtain the desired appearance, aroma, flavor, mouthfeel, and overall impression.

This brochure contains a partial list of measurable parameters. Should you require additional testing parameters please feel free to contact Shimadzu.



# Top Beer Analyses

In the average brewery, the brewing chemist needs to understand and address many quality assurance standards. Some of the major concerns are color, IBU (International Bittering Units), and alcohol content. The alcohol content on the label is required by legal organizations such as the USA's Alcohol and Tobacco Tax and Trade Bureau (TTB). At some point, calorie and carbohydrates content may also be required on alcohol labels.

Beyond these required analyses, there is great interest in other beer analytics. The regional water quality is known to greatly affect the flavor profile of beer, and when brews that were once consistent become troublesome, examination of the water can be enlightening. In this section, equipment for examining these factors affecting beer quality and production will be detailed. For more information about any of these products, please contact your Shimadzu representative.

## Color, IBU, & Alcohol Content



Shimadzu UV-1800 for measurement of beer IBU and color

One of the most useful pieces of equipment for a brewery's laboratory is the spectrophotometer. American Society of Brewing Chemists (ASBC) methods indicate at least 12 measurements, including beer bitterness (Beer-23) and beer color (Beer-10), that can be achieved using a single piece of equipment. For the purpose of a novice brewery lab, the most important measurements are IBU, color, and alcohol content. IBU or international bittering units is a measure of the bitterness of beer, derived from the alpha acids in hops. The color of beer ranges from pale to very dark and can be detected by the naked eye. However, a scale has been developed – degrees Lovibond – to define color intensity. There are conversion factors when switching between Lovibond, ASBC's Standard Reference Method (SRM), and the European Brewery

Convention (EBC). The strength of a beer is measured by volume and expressed as a percentage of alcohol (the number of milliliters of pure alcohol in 100 ml of beer). Bitterness and color can be performed on a UV-Visible spectrophotometer with built-in calculations. Alcohol is performed with a gas chromatograph (GC).

Examples of other beer and wort characteristics that can be measured by UV-Vis spectrophotometers include carbohydrates (Beer-41), copper (Beer-19), diacetyl (Beer-25 D), FAN or Free Amino Nitrogen (Wort-12 and Beer-31), iron (Beer-18A, C), oxygen (Beer-34), protein (Beer-11), total polyphenols (Beer-35), sulfur dioxide (Beer-21), wort color (Wort-9), and Thiobarbituric Acid Index (TBI by Wort-21).

Note: typical laboratory equipment such as chemicals, glassware, pipettes, shaker, centrifuges, etc. is not included.



# Color

The color of food and beverages is so important that it's been said that people "eat with the eyes" and their palates. If a food tastes bad, someone will probably not eat it again; however, if it doesn't look good it may never be tasted at all.

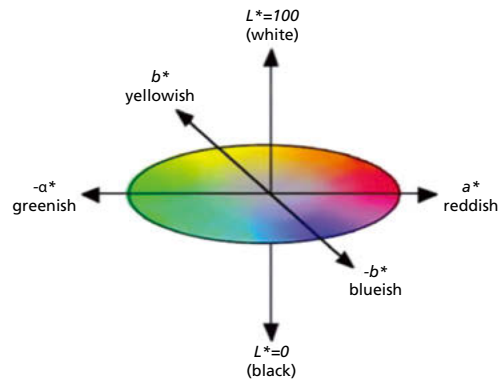
The color of beer can be measured with a UV-Visible spectrophotometer. There are two methods for measuring the color of beer depending on how much information is required. The first method is a single point measurement at 430 nm with a 1 nm bandwidth. This method has been reported to provide 92% of the information on beer color. Americans and Europeans perform the same measurement, but the color schemes are different. Americans use the Standard Reference

Method (SRM) while the Europeans use the European Brewery Convention (EBC). To convert from SRM to EBC, use the formula:  $EBC = SRM \times 1.97$ . The higher the SRM value, the darker the beer.

The second method, which provides more color information, is the CIELAB method, also called Tristimulus Analysis. This method measures 81 points at 5 nm increments from 380 nm to 780 nm. The color vision theory is based on three opponent pairs for the  $L^*a^*b^*$  values where the "L" is the luminosity, "a" is the red to green color component, and "b" is the yellow to blue color component. This type of measurement requires optional Shimadzu color software.



Shimadzu UV-1800 for measurement of beer color



CIELAB Color Space for Beer Analysis



# IBU

Bitterness is one of the major flavor components in beer and is used to balance out the sweetness from the malts. The bitterness from the hops is caused by alpha acids, which are extracted from the hops flower during the boil process. The alpha acids are converted to iso-alpha acids and provide the bitter taste. Hops also add their own aroma and flavor to the beer as well as provide antibacterial and preservative qualities. The International Bitterness Units (IBU) scale was created to measure the perceived bitterness of beer. The method requires a solvent extraction followed by measurement at 275 nm with a UV-Visible spectrophotometer. Another method for measuring

bitterness in beer is by measuring the iso-alpha acids (IAA). This process requires a different solvent extraction and measurement of absorbance at 255 nm. Results are reported in units of mg/L or parts per million (weight/volume). There can be some differences between the results of these two methods.

The European Bitterness Units (EBU) scale as defined by the European Brewery Convention (EBC) has a different process than the IBU scale of American Society of Brewing Chemists (ASBC).

For hops analysis by specific compound type, see "Section 2: Top Beer Analyses – Hop Degradation Analysis – Alpha Acids."



Shimadzu UV-1800 for measurement of beer bitterness and alcohol content



# Alcohol Content

Alcohol content, or strength of the beer, is expressed as a percentage, usually in terms of volume of ethanol divided by volume of beer. In many countries, ethanol concentration is required to be on the label by law. For example, in the U.S., the reported values must be within +/- 0.3% absolute of the true value.

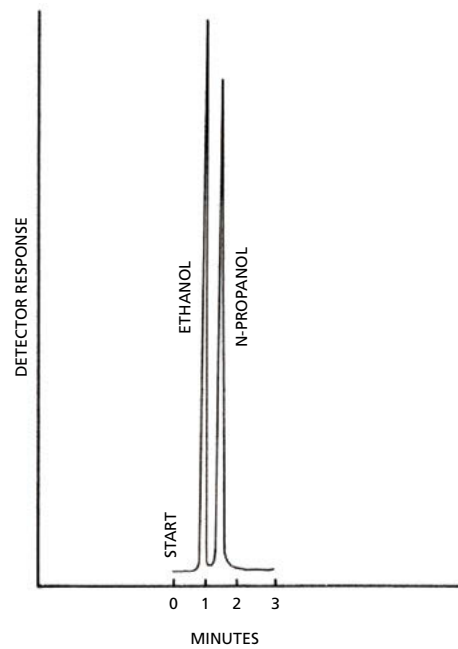
An accurate way to measure percent ethanol concentration in beer is by measuring the specific gravity of the distillate volumetrically or gravimetrically; however, some consider this method to waste beer, energy and, most importantly, time. A less accurate but easier method is to measure the specific gravities of the unfermented wort and fermented beer. The sugars in the unfermented wort raise the specific gravity as compared to water, while the alcohol lowers the specific gravity.

The differences assume the conversion of sugars to alcohol by the yeast. However, it should be noted that fermented beers contain some residual sugars, so the alcohol content is estimated.

Shimadzu's solution to measuring ethanol content is by gas chromatography (GC) with flame ionization detection (FID). The measurement can be performed according to AOAC Official Methods of Analysis 984.14 as well as by the American Society of Brewing Chemists (ASBC) as Beer-4D. The GC separates the ethanol from all other components in the beer so there are no interferences. Also, n-propanol is added to the beer and used as an internal standard during the analysis. The method injects two microliters of sample into the GC, and accurate analysis is completed in a couple minutes as shown below.



Shimadzu GC-2014 for measurement of alcohol content



Typical gas chromatogram for ethanol in beer with peak height response compared with that of n-propanol internal standard\*

A GC-2010 Plus or GC-2014 with the addition of a headspace autosampler can be used for analysis of diacetyl, as described in "Section 2: Diacetyl Levels," page 9.

\* From ASBC Beer-4D. Ethanol Determined by Gas Chromatography (GC)



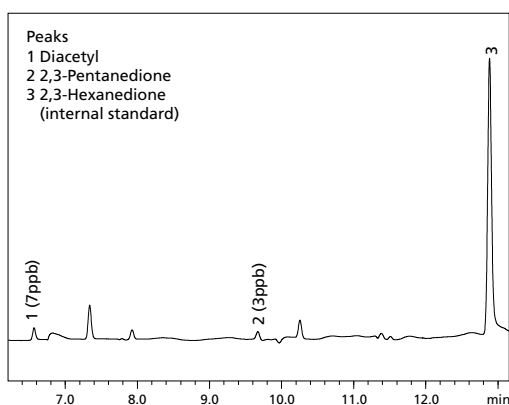
# Diacetyl Levels

Diacetyl (2,3-butanedione) and 2,3-pentanedione are in a class of compounds called vicinal diketones (VDKs). One of the biggest returns on investment (ROI) for instrument purchase consideration is how long the fermentation tanks are holding product before the brewing process is complete. This is checked by measuring the VDKs.

The VDKs give a buttery flavor, which is generally unwanted and a marker that the fermentation process is not complete. Using a liquid yeast, the primary fermentation process can take

8-14 days. Assuming an average of 12 days, the brewer could increase production by 10% a year by cutting the hold time in the fermentation tank by one day per batch. According to the European Brewery Convention (EBC), analysis is performed with a headspace GC with an ECD detector.

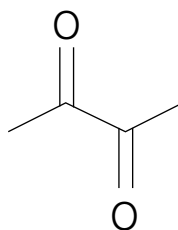
Note – The same GC-2014 with the addition of a FID detector can be used for analysis of alcohol content described in "Section 2: Alcohol Content," page 8.



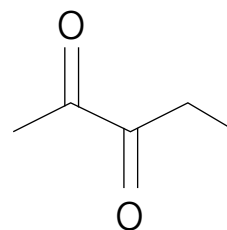
Vicinal diketones (VDK) in beer by Headspace GC



Shimadzu GC-2010 Plus or GC-2014 with HS-10 Headspace Autosampler for analysis of vicinal diketones



Diacetyl (2,3-butanedione)



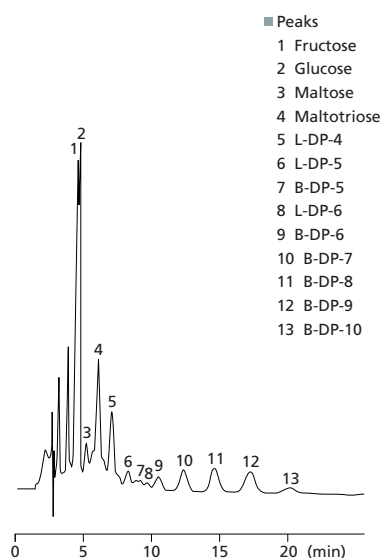
2,3-pentanedione

\* Shimadzu brochure: ECD-2010 Exceed, C180-E080, Page 6

# Carbohydrates

There are four major classes of carbohydrates. These include monosaccharides, disaccharides, oligosaccharides, and polysaccharides, where the prefixes mean one, two, few, and many sugars, respectively. Monitoring these sugars is an indication of where the fermentation process is at during the brewing process.

Shown is a chromatogram of the analysis of monosaccharides

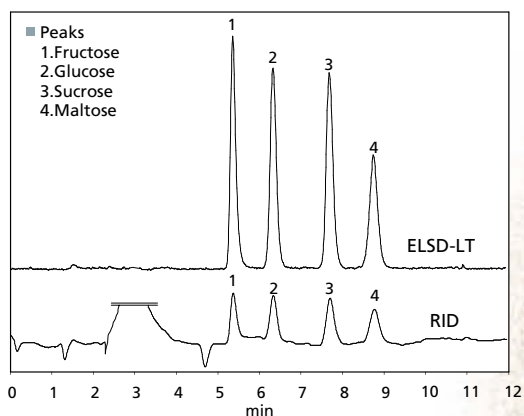


Analysis of Carbohydrates in Beer

(e.g. glucose) up to 10 sugar oligosaccharides called deca-saccharides (e.g. B-DP-10)\*. The method requires an HPLC with a Refractive Index Detector (RID). Generally, RID is more than sensitive enough; however, a low-temperature evaporative light scattering detector (ELSD-LT) can provide even more sensitivity as in the example below\*.



Prominence HPLC with RID-20A for carbohydrate analysis



Example of comparison of sensitivity between an ELSD-LT and RID for sugars\*



\* <http://www.shimadzu.com/an/industry/foodbeverages/e801ci00000006r9.htm>  
 \* Shimadzu Application News No.L294: "Application of Evaporative Light Scattering Detector ELSD-LT (Part 2) Analysis of Oligosaccharides in Beer"

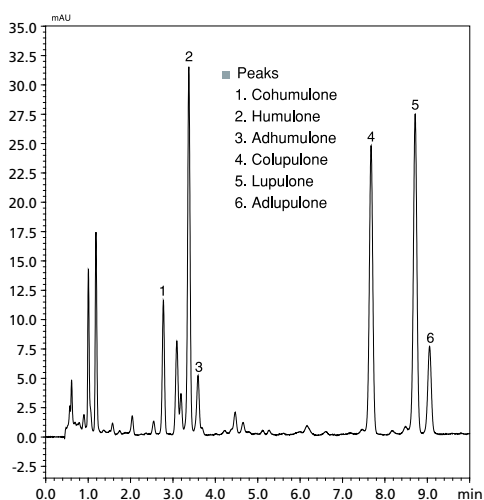
# Hop Degradation Analysis – Alpha Acids

Hops, a major ingredient in beer, contain components called alpha acids (humulones) and beta acids (lupulones). Through the brewing process, alpha acids are converted to iso-alpha acids (isohumulones), which are bitter components of beer. Beta acids are said to influence the bitterness balance.

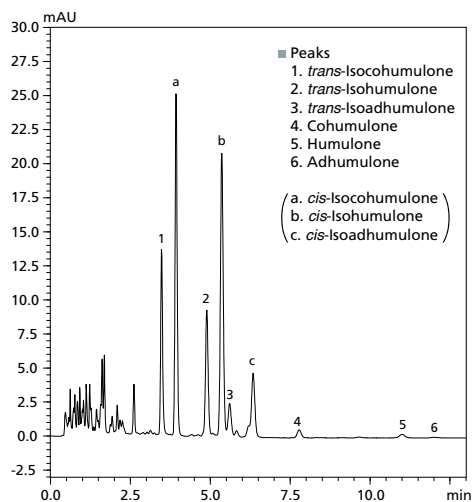
In this example, the left chromatogram is for the alpha acids and beta acids in hop pellets before the brewing process\*, whereas the right chromatogram shows the analysis of alpha acids and iso-alpha acids in beer after the brewing process# utilizing the Nexera XR UHPLC with SPD detection.



Nexera XR UHPLC



Analysis of alpha acids and beta acids in hop pellets by Nexera XR system\*



Analysis of alpha acids and iso-alpha acids in beer by Nexera XR system#

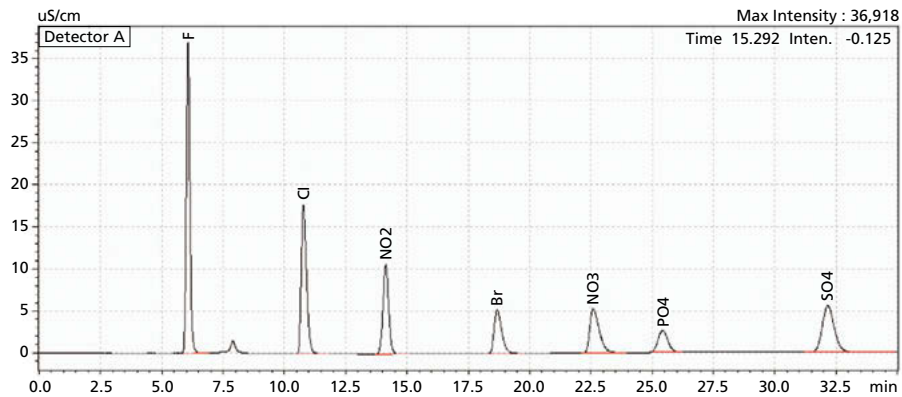
\* Shimadzu Application News No.L389  
# Shimadzu Application News No.L397

# Water Characteristics

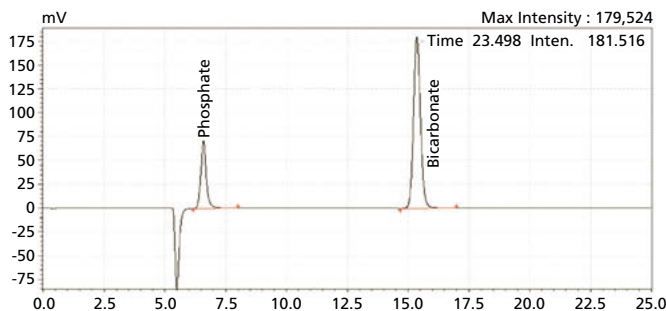
Water makes up approximately 90% of beer ingredients. So it is easy to understand why regional influence can have an effect on beers. Regions where rain falls in rivers, lakes, streams and ultimately reservoirs with impermeable hard rock will likely produce soft water with low dissolved salts. On the other end of the spectrum are areas with permeable rock such as limestone (calcium carbonate) and gypsum (calcium sulfate), which will accumulate many minerals on the way to the reservoir. Examples of regions known for specific beers include pales ales of Burton, England, where water has higher levels of gypsum; porters, associated with another part of England, more specifically London; hard water stouts such as Guinness contain vitamins and minerals, especially vitamin B and probiotics, and are identified with Dublin, Ireland; and pilsners, a blond lager connected with soft water from the Pilsner Urquell Brewery, Czech Republic. Since water for different cities or municipalities has different characteristics, it is up to the brewmaster to determine if some

of these minerals should be added or removed before the process begins. A good place to start on the determination of mineral concentration are free reports from local municipalities. If the quality of water is not suitable, then some filtration may be required. Hard water has high concentrations of calcium and magnesium carbonates. Other carbonate ions that affect beer flavor are sodium, chloride, and sulfate. These can all be analyzed by ion chromatography.

The analysis of fluorine (F), chlorine (Cl), bromine (Br), nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>), and sulfate (SO<sub>4</sub>) can be performed according to USEPA method 300.1\*. Shown below is the analysis of carbonates and phosphate (PO<sub>4</sub>)\*. Analysis of ammonia (NH<sub>4</sub>) can be found in the same application note\*. Also, note that calcium, magnesium, phosphorus and sodium can be analyzed by AA, ICP, and ICP-MS as discussed in the section "Troubling Shooting your Beers – Inorganic Water Contamination," page 16.



Ion chromatography analysis of drinking water according to USEPA 300.1\*



Ion chromatography analysis of phosphate and bicarbonates\*



Ion Chromatography System

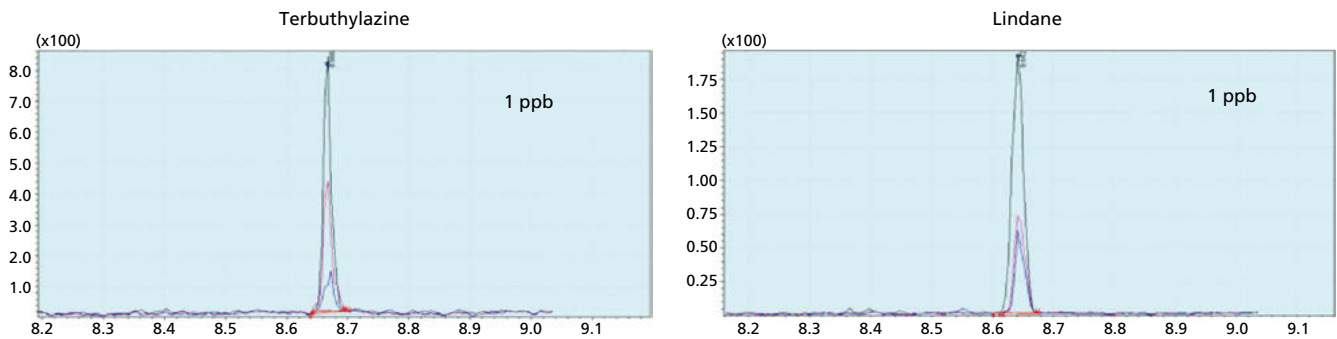
\*Shimadzu Scientific Instruments, SSI-LC-06-2015: "Ion Chromatography Guide"

# Troubleshooting your Brews Ingredient Analysis – Hops Contamination

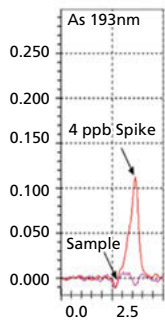
Hops provide the bitterness in beer. They originate from compounds, such as isohumulones, which are part of a class of compounds referred to as iso-alpha acids. Iso- $\alpha$ -acids,  $\alpha$ -acids and  $\beta$ -Acids were discussed in more detail under the section of "Top Beer Analyses – Hop Degradation Analysis – Alpha Acids," page 11.

Organic compound contaminants such as pesticides are a major concern due to their potential for causing health-related illnesses. In a recent communication\*, 34 pesticides from five

classes of compounds, including organonitrogen, synthetic pyrethroids, organochlorines, organophosphorus, and carbamates, were analyzed in hops. Samples were prepared by the QuEChERS method before introduction into a GCMS-TQ8040 triple quadrupole mass spectrometer. Shown are two examples of pesticides at a 1 ppb level. The pesticides include the toxic herbicide Terbutylazine and the insecticide Lindane, a known carcinogen and neurotoxin.



Overlaid MRM chromatograms for two pesticides at 1-ppb in hops by GCMS-TQ8040 triple quadrupole mass spectrometer



Analysis of hops at 4 ppb arsenic (As) by AA-7000



Shimadzu GCMS-TQ8040 Triple Quadrupole Gas Chromatograph Mass Spectrometer

Before hops can be used they should be checked for inorganic and organic contaminants. The inorganic contaminants can come from the plants absorbing heavy metals, such as Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb), from the soils. Shown here is an example of arsenic analysis at four parts per billion in hops# measured by an AA-7000 atomic absorption spectrophotometer.

\* Pittcon 2015 Presentation: "Screening Medical Marijuana for Pesticides by GC-MS/MS" where hops were used in place of marijuana.

# Shimadzu Scientific Instruments Application News No. AA-004: "Analysis of "The Big Four" Heavy Metals in Hops by Electrothermal Atomization and Cold Vapor

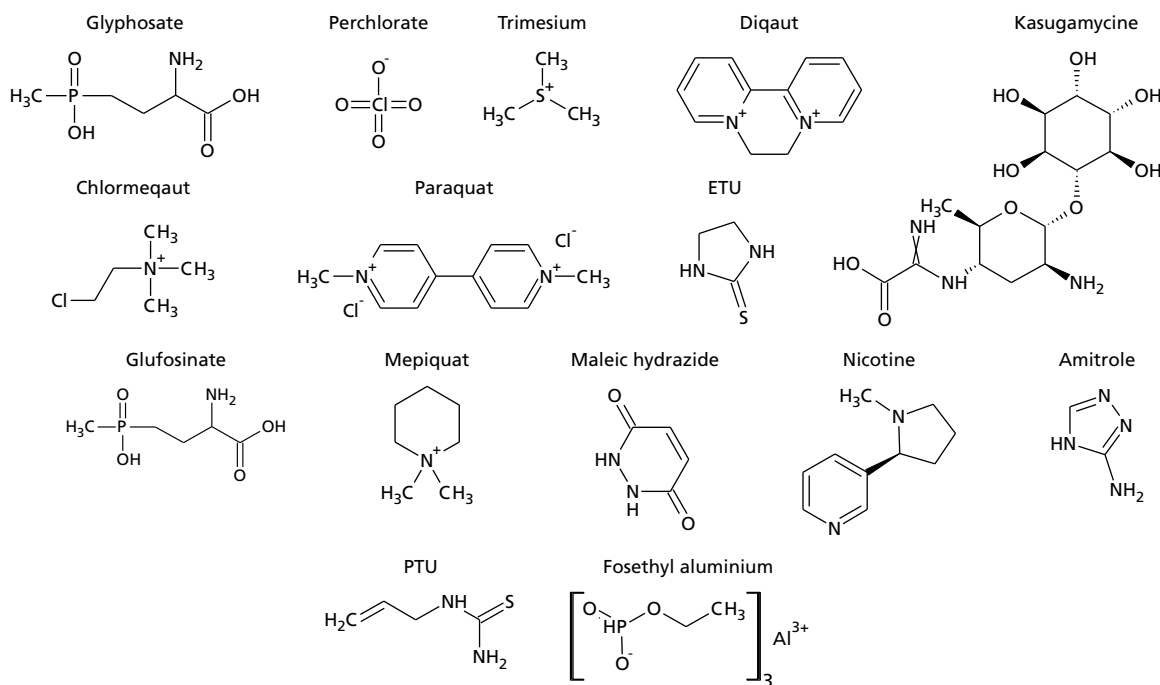


# Ingredient Analysis

## Hops and Organic Water Contamination

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. This example shows results for the quantitative determination of glyphosate, glufosinate and AMPA (aminomethylphosphonic acid) using the LCMS-8050 triple quadrupole LC/MS/MS system according to DIN 38407-22 of the German standard methods for the examination of water, waste water and sludge. This instrument was used instead of HPLC with fluorescence detection. The LCMS-8050 allows the determination of glyphosate in concentrations as low as 0.1 µg/L. Sample preparation was done after derivatization with 9-fluorenylmethyl chloroformate (FMOC-CL).



Shimadzu LCMS-8050 Triple  
Quadrupole Mass Spectrometer

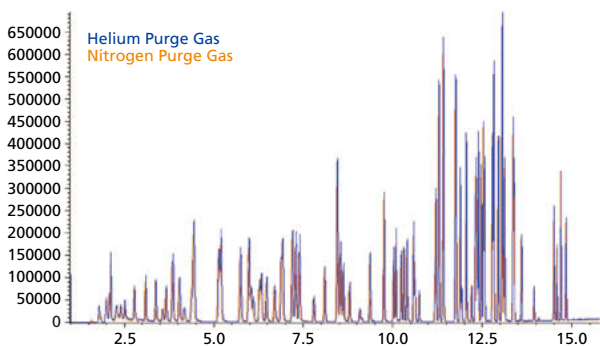


Application News "Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LCMS/MS"

# Ingredient Analysis – Organic Water Contamination

Many common organic water contaminants affecting beer flavor are organic volatile and semi-volatile compounds such as naphthalene, hexachlorobenzene, and hexachlorocyclopentadiene. To analyze these and other specific organic compounds in water, follow the purge and trap GCMS

method USEPA 524 for volatiles\* and the liquid injection method USEPA 525 for semi-volatiles. The chromatogram below shows the analysis of 76 volatile compounds from 0.5 ppb to 20 ppb. Some countries permit headspace to replace purge and trap methods.



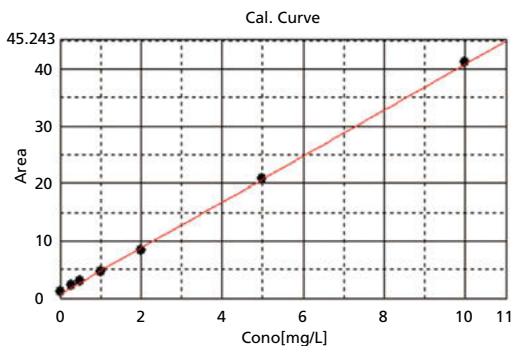
Overlay of 20 ppb volatile standards purged in helium and nitrogen following USEPA method 524



Shimadzu GCMS-QP2010 SE Gas Chromatography Mass Spectrometer for the Analysis of Volatile and Semi-volatile Compounds

For non-specific organic contaminants, the TOC-L Total Organic Carbon analyzer can provide analysis for total organic carbon, total inorganic carbon, and total carbon by EPA Method 415.3<sup>#</sup>. The 7-point calibration curve shown below includes standards at

0, 0.3, 0.5, 1, 2, 5, and 10 ppm with a coefficient of determination ( $r^2$ ) of 0.9996. The TOC-L has an optional accessory for measuring Total Nitrogen (TN).



TOC calibration curve from 0-10 ppm in drinking water



Shimadzu TOC-L Total Organic Carbon Analyzer for the Analysis of Non-specific Carbon

\* Shimadzu Scientific Instruments Application News No. GCMS-1502: "Guide to US EPA Methods 524.3 and 524.4 for Analysis of Volatile Organic Compounds in Drinking Water"  
<sup>#</sup> Shimadzu Scientific Instruments Application News No. TOC-004: "EPA Method 415.3 - Determination of TOC in Source Water and Drinking Water"

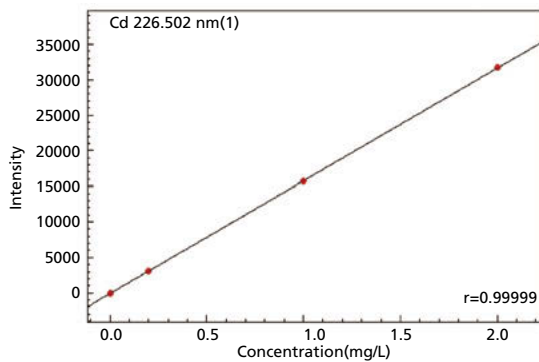
# Ingredient Analysis – Inorganic Water Contamination

Water, the universal solvent, is required to be free of contaminants and odors that will affect the beer's flavor, color, and taste. Heavy metal or inorganic contaminants, such as Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb), can be harmful to one's health. These and other metals can be analyzed by atomic absorption (AA-7000), inductively coupled plasma emission (ICPE-9800), or inductively coupled plasma mass spectrometry (ICPMS-2030) according to EPA methods 200.9,

200.7, and 200.8, respectively.

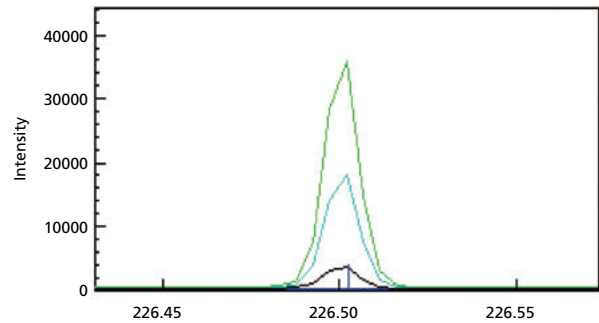
Shown in the graph is a 0, 0.2, 1 and 2 µg/L calibration curve for cadmium as well as the spectral line profile on an ICPE-9800.

The above techniques can also measure approximately 70 elements, such as aluminum, calcium, chromium, copper, iron, magnesium, nickel, phosphorus, potassium, and sodium. Salts such as sodium can have an effect on the pH, precipitation, wort, and yeast.



Calibration curve of cadmium in water at 0, 0.2, 1, and 2 µg/L by ICPE-9800

Cd 226.502  
Cond 1



Spectral line profile of cadmium in water at 0, 0.2, 1, and 2 µg/L on ICPE-9800



Shimadzu's ICPE-9800 Inductively Coupled Plasma for the analysis of metals

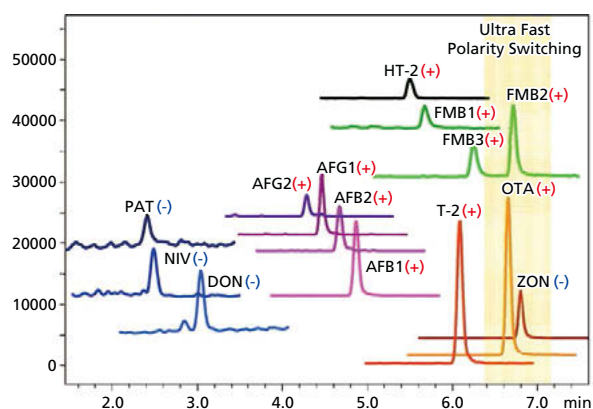
# Ingredient Analysis – Mycotoxins in Beer Grains

Mycotoxins often exist as contaminants in grains. To ensure consumer food safety, manufacturers of food and beverages have to strictly manage risks from such contaminants. To maintain high-quality food standards, it is essential to rapidly determine the concentrations of hazardous mycotoxins in foods or beverages.

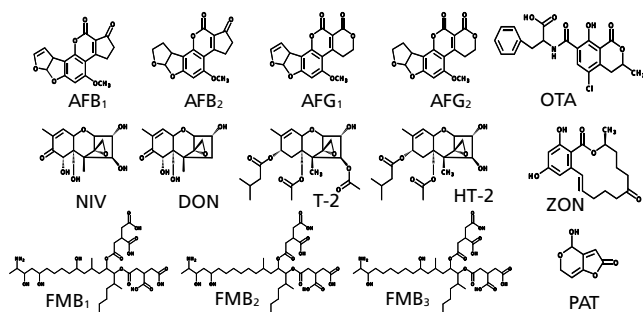
UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. In this study, a high-throughput method for

the quantification of 14 mycotoxins in beer was developed\*.

The 14 mycotoxins: patulin (PAT), nivalenol (NIV), deoxynivalenol (DON), aflatoxin (AF) B1, B2, G1, G2, T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZON), fumonisin (FM) B1, B2, B3 and ochratoxin A (OTA) were determined in beer by LC-MS/MS using a UHPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer with fast polarity switching and high-speed scanning.



Analysis of 14 mycotoxins in beer at 10 ppb by UHPLC-MS/MS\*



Structure of 14 Mycotoxins



Shimadzu Triple Quadrupole LCMS-8050

\*ASMS 2012 Poster WP27-580: "High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS"; work performed using the previous model, LCMS-8030

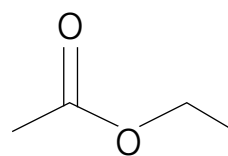
# Brewing Chemistry Analysis – Esters & Aldehydes

Esters provide the fruity flavor found in ales and wheat beers. However, in lagers, esters may be considered undesirable. Volatile organic compounds such as esters, aldehydes, and alcohols are formed through the fermentation process. Esters are formed through a process called esterification in which an alcohol (i.e. ethanol) and an acid react to form such compounds as ethyl acetate and isoamyl acetate. Ethyl acetate can have flavor characteristics similar to that of a pear at low concentrations or a solvent-like flavor at higher concentrations. Isoamyl acetate provides a banana-like flavor found in beers. Ethyl acetate and isoamyl acetate are produced from the reaction of ethanol and isoamyl alcohol, respectively, with acetic acid. The amount of esters produced is mainly controlled by the type of yeast used. Yeast contains an enzyme called alcohol acetyl

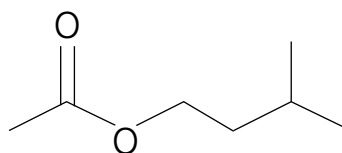
transferase (AAT) which is mainly responsible for ester production during fermentation. Other methods for controlling esters include fermentation at a higher temperature, which increases yeast growth and, in turn, AAT, resulting in a higher concentration of esters. Similarly, if the yeast is under pitched, yeast will grow quickly, thus increasing the AAT and boosting the ester concentration. Also, under oxygenation of the wort will result in higher levels of esters in the beer. Aldehydes such as acetaldehyde are also a byproduct of the fermentation process, providing flavor and aroma similar to that of green apples. Analysis of ethanol, esters, aldehydes, and other volatile compounds in beer is performed by headspace gas chromatography (GC) with a flame ionization detector (FID).



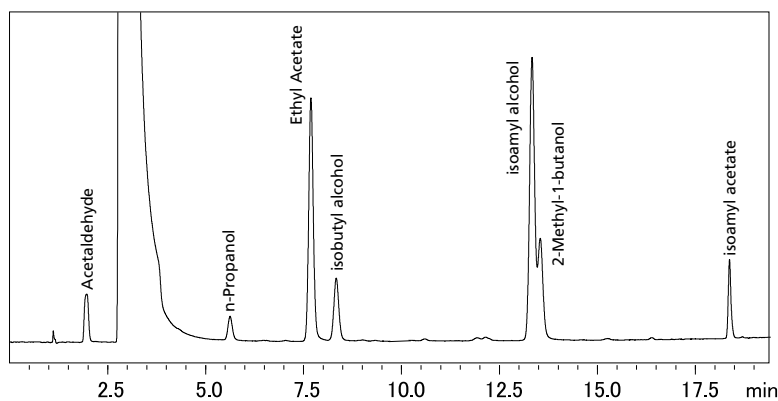
Shimadzu GC-2010 Plus or GC-2014 with HS-10 Headspace Autosampler for analysis of esters in beer



Ethyl acetate



Isoamyl acetate



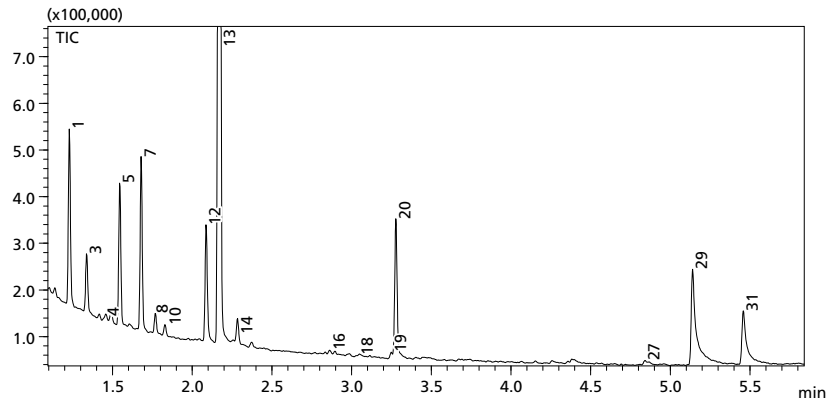
Chromatogram of esters, acetates, and alcohols by headspace GC-FID



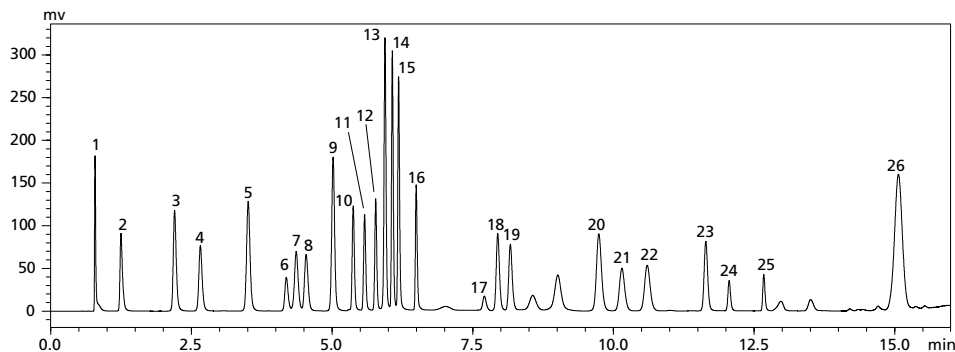
# Brewing Chemistry Analysis – Amino Acids

Generally, there are two methods for a beer's color, not including using additives. One is by the Maillard Reaction in which an amino acid and a reducing sugar go through a chemical reaction. This is the same reaction as browning meat or toast. The other reaction is a caramelization process where

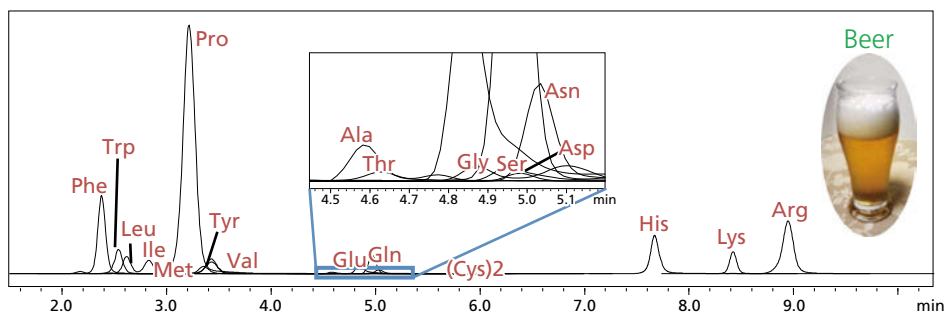
sugars decompose through pyrolysis. These reactions not only add color, but also add flavor to the beer. Thus, when brewing beer, it is important to know the free amino acids available. There are a number of methods for analyzing amino acids and below is a sampling by GCMS, UHPLC, and LC-MS/MS.



Total ion current chromatogram for 31 amino acid derivatives in beer by GCMS-QP2010 Ultra\*



Chromatogram of 23 amino acids using pre-column derivatization by Nexera UHPLC with SIL-30AC Autosampler and RF detection\*



Analysis of 20 amino acids in beer without derivatization by LC-MS/MS<sup>§</sup>

\* Shimadzu GCMS Application Data Sheet No.1: "Analysis of Amino Acids Contained in Alcohol"

\* Shimadzu Application News No. L437: "High Speed, High Resolution Analysis (Part 45) Analysis of Pre-Column Derivatized Amino Acids by the Nexera SIL-30AC Autosampler (Part 2)"

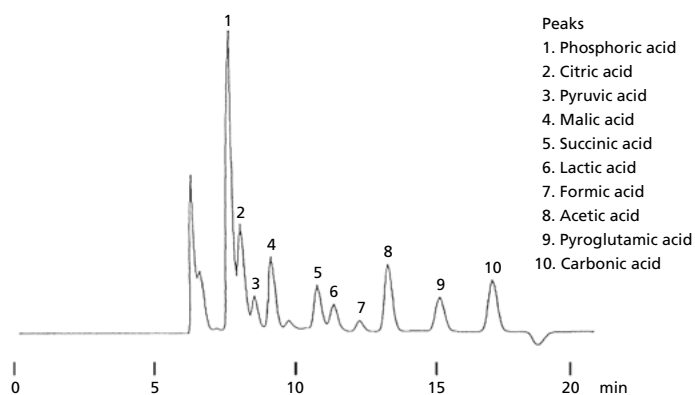
§ ASMS 2014 Poster TP-510: "Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS"

# Brewing Chemistry Analysis – Organic Acids

Organic acids, along with amino acids and sugars, are important substances in biochemistry. They are major analytical target components in various fields such as food products. They are particularly important components in determining the fragrance and flavor of food products. This report\* explains the basic principles of the Prominence Series Organic Acid Analysis System

that is based on the post-column pH-buffered electroconductive detection method.

This example indicates that these organic acids can be analyzed without being affected by impurities, even without any special sample pretreatment.



Analysis of beer for organic acids by Shimadzu HPLC Prominence Organic Acid Analysis System



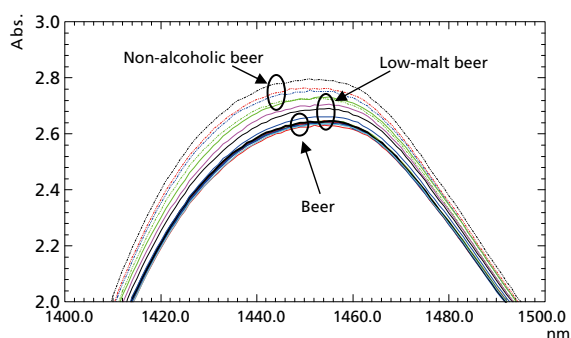
Prominence HPLC with Electroconductive Detection for organic acid analysis

\* Shimadzu HPLC Application Report No. 25, C190-E105: "Principles and Applications of the Prominence Organic Acid Analysis System"

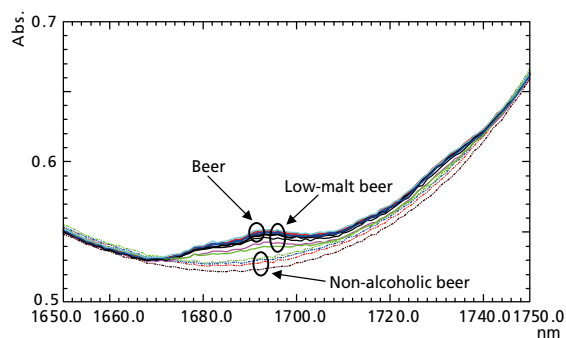
# Classification of Beer Types

The market is now bustling with beverages such as non-alcoholic beer and low-malt beer with beer-like flavors. Beer, low-malt beer, and non-alcoholic beer can all be considered types of beer. A great many varieties have been produced and marketed with modifications to the ingredients to adjust such characteristics as alcohol and calorie content. The alcohol content in the samples is: beer: 5 % and above; low-malt beer: 3 – 5.5 %; non-alcoholic beer: 0 %. The protein

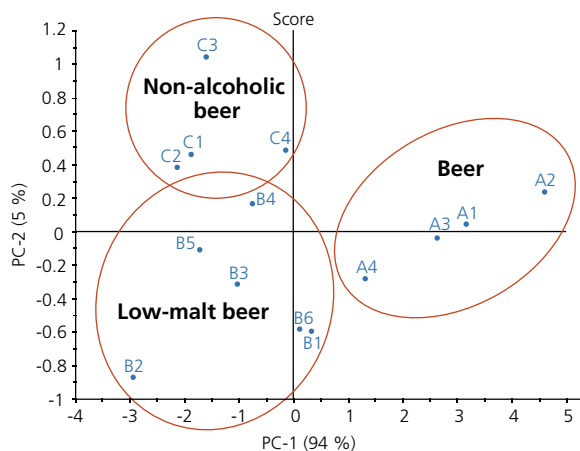
content per 100 mL is: beer: 0.2 – 0.4 g; low-malt and non-alcoholic beer: 0 – 0.3 g. The amount of absorption in the ultraviolet and near infrared regions in these data approximately reflect the given content values for protein and alcohol, respectively. The absorption spectra of 14 types of commercially available beers (4 types of beer, 6 types of low-malt beer, and 4 types of non-alcoholic beer) were measured using the Shimadzu UV-3600.



Non-alcohol beers have a higher water peak near infrared wavelength of 1450 nm



Higher alcohol beers have a higher ethanol peak near infrared wavelength of 1695 nm



Classification of beer type by multivariate analysis conducted by principal component analysis (PCA)



UV-3600 Plus for Analysis of Classification of Beer Types

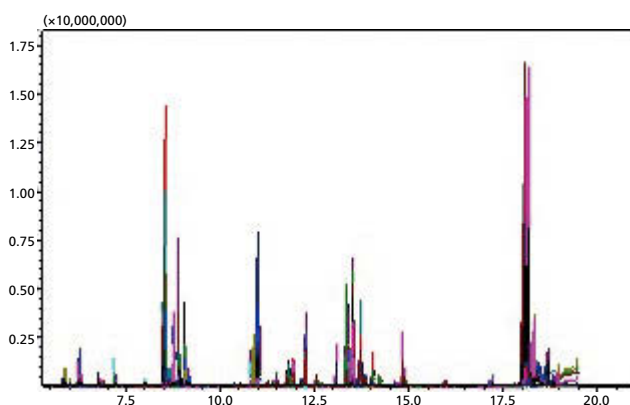
# Classification of Beer Types #2 – Metabolomics

Nowadays, “forecast of taste” or “visualization of food additional values” have been gaining the momentum in food industry, that is conducted by using analysis equipment in many cases.

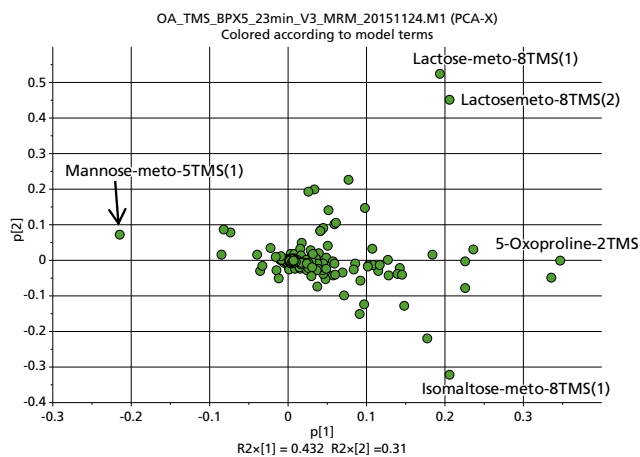
This time, five beers (lager beer, lager beer containing rice, ale beer, low-malt beer, and non-alcoholic beer) were analyzed for classification. Shimadzu GCMS-TQ8040 and LCMS-8060 were used for the analysis of hydrophilic metabolites in the beers that could strongly affect taste.

Analysis by Shimadzu GCMS-TQ8040 and Smart Metabolites Database

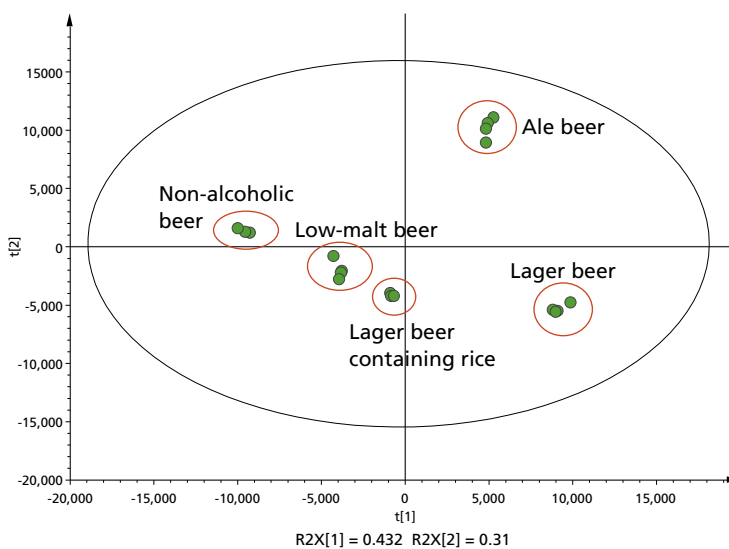
Targeting 475 components by GC-MS/MS, we can detect about 300 components in each five beers. By the principal component analysis (PCA) from the results, the differences among beers can be examined in the score plot. Besides, we can confirm from the loading plot that some sugars and so on are the strong relation to the differences in the score plot.



MRM Chromatogram



Loading plot

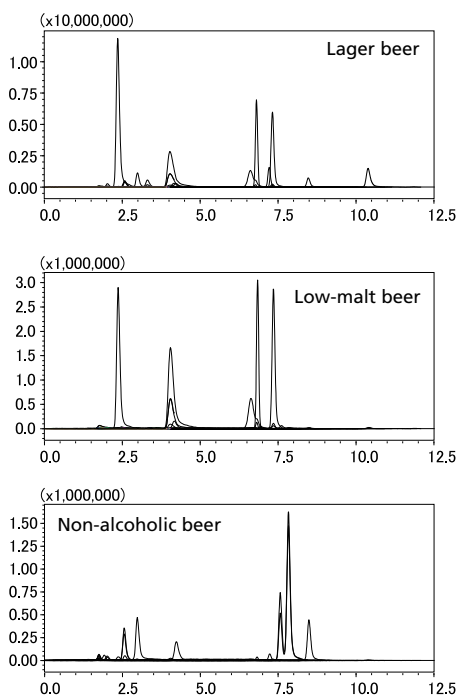


Score plot

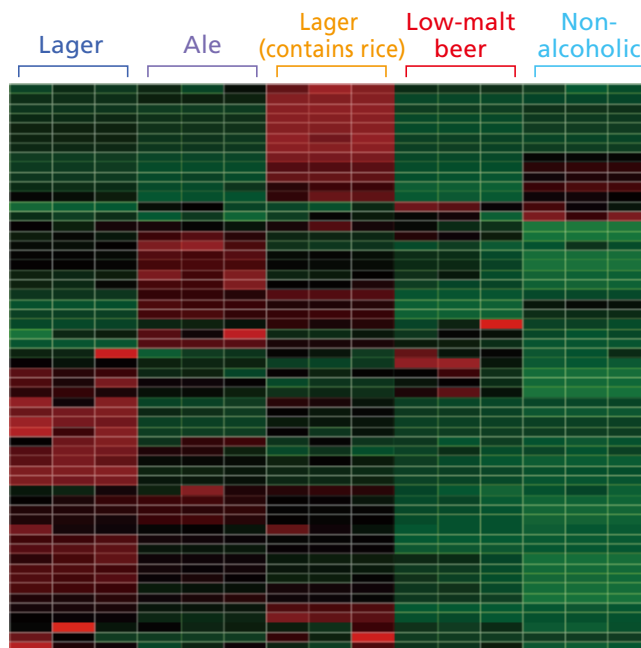
Score plot :Classification of beer type by multivariate analysis conducted by principal component analysis (PCA) from the result of GC-MS

Analysis by Shimadzu LCMS-8060 and Method Package for Primary Metabolites  
 Targeting 97 components by LC-MS/MS such as amino acids, organic acids, nucleic acid associated materials, and so on, we can detect 58 components in each five beers. The hierarchical clustering analysis was done for those 58 components by

Traverse MS software (Reifycs Inc.) . From the result of the hierarchical clustering analysis, five beers can be classified by differences of components contained in each beer , and also the similarity among each beer can be confirmed by differences of contained amount.

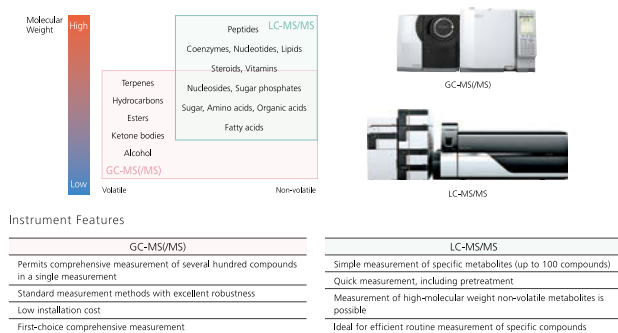


MRM chromatograms



Classification of beer type by hierarchical clustering analysis (HCA)

### Selection of the appropriate instrument for quantitative metabolomics



GC-MS/MS and LC-MS/MS were used for the analysis of hydrophilic metabolites in the beers. GC-MS/MS and LC-MS/MS are widely used for measuring the total amount of metabolites in a sample. Selection of the appropriate instrument to be used is based on the target compounds and the objective.

\*12th Annual Conference of the Metabolomics Society Poster #221: "Mass spectrometry-based metabolomics to differentiate beer types"

\*ASMS 2016 Poster ThP 445: "Metabolomic profiling of beer types by widely-targeted LC/MS measurement"

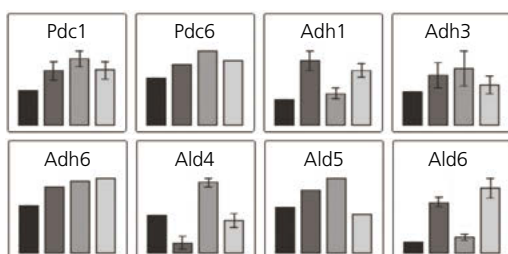
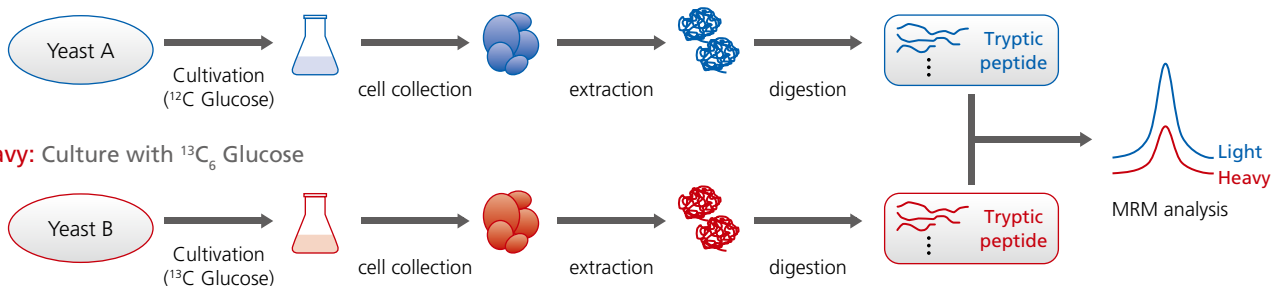


# Brewing Chemistry Analysis – Profile of Metabolic Enzyme

Material production using microorganisms such as *Saccharomyces Cerevisiae* has been applied to fermented food such as beer. These microbial cell factories have been metabolically engineered by regulating the expression levels of enzymes. To understand these metabolic pathways, it is important to monitor the abundance of enzymes. Metabolic enzymes can be subjected to trypsin digestion, and representative tryptic peptides selected for analysis. LC-MS/MS is a highly sensitive and specific analytical technique used for quantitation of specific peptides in biological samples. Unlike ELISA, LC/MS-based MRM (Multiple Reaction Monitoring) approaches do not require an antibody for each protein/peptide.

Yeast strain S288C and single gene deletion mutants of strain BY4742 were cultured in media containing non-labeled or C-13 labeled glucose for metabolic enzyme analysis. Strain S288C grown in heavy C-13 labeled glucose acted as an internal standard for comparison to the light C-12 mutated growth strains\*. Information regarding glycerol biosynthesis, ethanol pathway, tricarboxylic acid (TCA) cycle, polysaccharides, and amino acids was determined by LC-MS/MS analysis. In one example, the LCMS-8050 measured 303 peptides of 137 proteins by monitoring 2856 MRM transitions#. The library\* for metabolic enzymes in yeast contains 3,584 MRM transitions, including stable isotopes. It covers all 498 trypsin digested peptides of 228 types of enzymes derived from budding yeast.

## Light: Culture with $^{12}\text{C}_6$ Glucose



Metabolic profile of Ethanol pathway enzyme levels in wild type (black) and three deletion strains (greys) of *S. cerevisiae*.#



Shimadzu LCMS-8050 for measuring metabolic enzymes in yeast

\* Shimadzu Brochure C146-E275: "LC/MS/MS MRM Library for Metabolic Enzymes in Yeast"

# Shimadzu Corporation Application Data Sheet: "Quantitative proteomics of metabolic enzymes in *S. cerevisiae* with UFMS technologies"

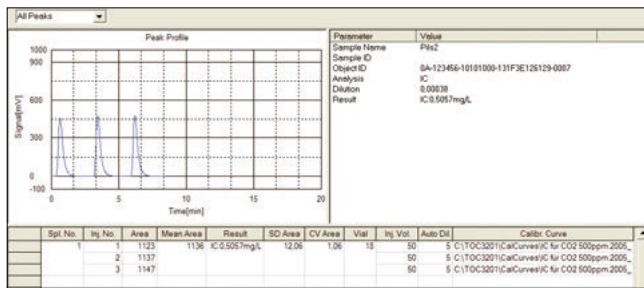
# Quality Assurance – CO<sub>2</sub> Determination

Quality assurance is only one of the attributes for brand name products such as beer. And quality is dependent on the quality of the starting materials, strict production processes as well as specialists whose keen eyes oversee the entire production. Finally, millions of consumers are testing their favorite brand of beer every day.

An important player in the production process and a condition for quality is invisible: carbon dioxide (CO<sub>2</sub>), formed during the fermenting process. After filling the beer into barrels and

bottles, carbon dioxide ensures that the necessary pressure is maintained. This is an important factor for guaranteeing shelf life and fresh-tasting beer. A constant concentration of carbon dioxide also ensures a steady taste and consistent quality of the beer. Beer contains 4 – 6 g/L CO<sub>2</sub>.

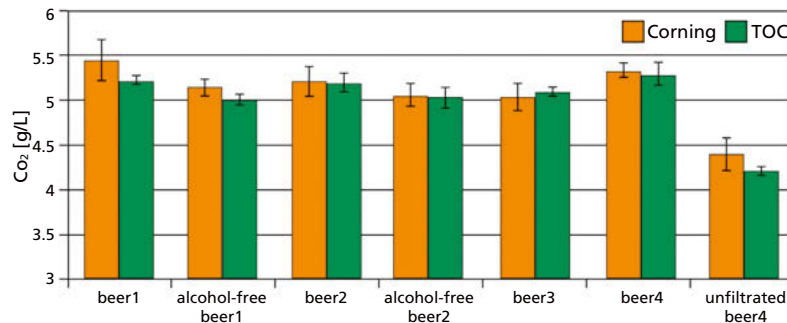
Carbon dioxide in the world of Total Organic Carbon (TOC) analyzers is referred to as Inorganic Carbon (IC). The IC method is a suitable alternative to the classical Corning reference method for measuring CO<sub>2</sub>\*.



Inorganic Carbon (IC) measured as CO<sub>2</sub> in beer by a TOC analyzer\*



Shimadzu TOC-L Total Organic Carbon analyzer for the analysis of CO<sub>2</sub>



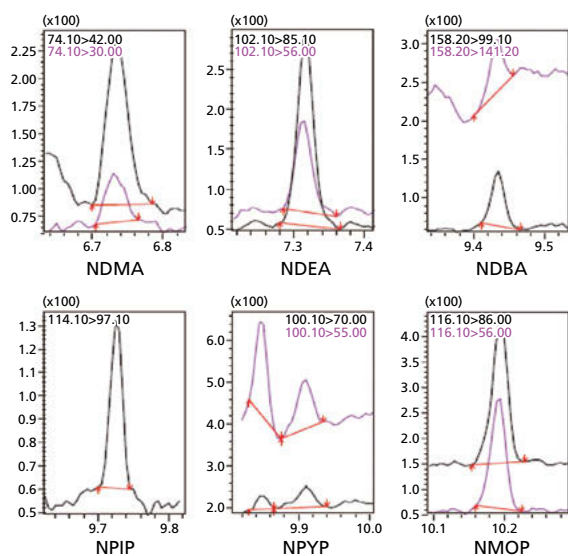
CO<sub>2</sub> concentration measured by TOC and Corning reference method for various beers\*

# Brewing Chemistry Analysis – N-Nitrosamines

Most N-nitrosamines are known to be carcinogenic and mutagenic. Consumption of nitrosamines, e.g., N-nitrosodimethylamine (NDMA), was reported to be a cause of gastric cancer, liver cancer, glioma and blood disorder. N-nitrosamines can be formed by a reaction between amine and nitrite under heating conditions in food processing. They have been found in malt-derived beverages like beer at trace levels. The presence of NDMA in malt and beer was first reported in 1974. Its concentration in malt depends on the drying techniques used. According to US and EU regulations, the

amount of nitrosamines in beer must be controlled to the acceptable levels, typically at 0.2 ~ 5.0 ppb depending on the country. The main N-nitrosamine that is monitored in malt and beer is NDMA.

Shown is a GC-MS/MS method using Multiple Reaction Monitoring (MRM) mode for simultaneous detection and quantification of six N-nitrosamines, including NDMA, for enhanced selectivity and sensitivity from the potential matrix interferences in beer samples\*.



MRM transitions of six N-Nitrosamines at 0.1 ppb in Beer by GC-MS/MS



Shimadzu GCMS-TQ8040 triple quadrupole mass Spectrometer

\*ASMS 2013 Poster TP738: "Method Development for Trace Level Detection of N-Nitrosamines in Beer by GC-MS/MS"

# Packaging Testing for Beer

When beer is not ready for direct consumption, then there must be some type of packaging such as cans, bottles, or kegs. For cans, they usually have a pull-tab. The question becomes how easy or hard should the pull-tab be to open. If the pull-tab opens too easily, then there is a possibility of leakage during shipping and handling. If the pull-tab is too hard to open, then the tab may break off before the can is opened. This may lead to people trying to puncture the can with a "tool" which can

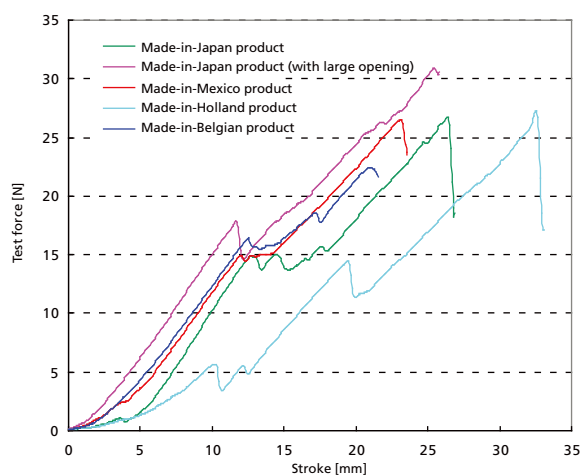
result in injury.

The Shimadzu EZ-X tensile testing system tests force versus stroke distance according to standard method JIS S 0022:2001.

The test requires to hold the can by hand; however, in the example below, a fixing jig was used to hold the can in position for more accurate testing results. A string is hooked on the pull-tab and it is pulled straight up at a speed of 500 mm/min.\*



Tensile test on pull-tab on beer can containers with EZ-X



Relationship between the Force and Stroke

Sample	Maximum Opening Strength [N]
Canned beer (made in Japan)	26.8
Canned beer (made in Japan, with a large lip)	30.8
Canned beer (made in Mexico)	26.5
Canned beer (made in Holland)	27.2
Canned beer (made in Belgium)	22.4

Force required to open beer from various countries\*

\*Source: Application Data Sheet #25: "Tensile Test on Pull-Tab Containers (Reference Standard: JIS S 0022:2001)"

# Equipment Packages

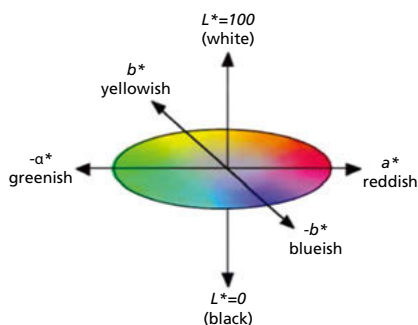
The next few pages will provide insight into choosing the right analytical instruments for a startup brewers lab, and for expanding the laboratory's capabilities to a midsize and, ultimately, high-end laboratory.

## Setting Up Your Lab

This package should enable you to determine Color and IBU.\* ASBC states that other possible beer measurement parameters are possible with a UV-Vis spectrophotometer, including carbohydrates (Beer-41), copper (Beer-19), diacetyl (Beer-25 D), FAN or Free Amino Nitrogen (Wort-12 and Beer-31), iron (Beer-18A, C), oxygen (Beer-34), protein (Beer-11), total polyphenols (Beer-35), sulfur dioxide (Beer-21), wort color (Wort-9), and Thiobarbituric Acid Index (TBI by Wort-21).

### UV-1800 UV-Visible Spectrophotometer for Beer Analysis

P/N	Model	Notes
220-92961-01	UV-1800	PC not required
206-67449-00	Optional Color Software for CIELAB Color	Requires PC & Monitor



### Analytical Balances for Lab Analysis (optional)

P/N	Model	Capacity (oz)	Capacity (g)	Minimum Display (mg)
321-71000-32	ATX84	3	84	0.1
321-71000-33	ATX124	4.2	120	0.1
321-71000-34	ATX220	7.8	220	0.1

### Top Loading Balances for Beer Ingredients (optional)

P/N	Model	Capacity (lb)	Capacity (kg)	Minimum Display (g)
220-93025-01	BX12KH	26.5	1.2	0.1
220-93025-02	BX22KH	48.5	22	0.1
220-93025-03	BX32KH	70.5	32	0.1
220-93025-04	BX32KS	70.5	32	1
220-93025-05	BX52KS	114.5	52	1



\*Note: typical laboratory equipment such as chemicals, glassware, pipettes, shaker, centrifuges, etc. are not included. Also note optional GC method can determine alcohol content.

# Expanding Your Lab

This package enables you to determine alcohol content, carbohydrates, alpha acid levels, diacetyl or VDK levels, organic water contaminants, hops contamination, esters, aldehydes, n-nitrosamines, amino acids, and organic acid levels utilizing triple quadrupole GC-MS/MS and UHPLC.\*

This section assumes you have all of the analytical instruments

under "Section 4: A. Setting Up Your Lab" in order to analyze beer color and IBU content by UV-Visible spectrophotometry. The goal of instruments on this page is to increase the types of testing for different parameters in order to improve quality, create new products, or conduct research and development.



Shimadzu GCMS-TQ8040 Triple Quadrupole Gas Chromatograph Mass Spectrometer with headspace for analysis of alcohol content, diacetyl levels, organic water contaminants, hops contaminations, esters, aldehydes and amino acids. Alcohol content can also be analyzed by GC.



The Nexera X2 UHPLC can be used to analyze for carbohydrates, alpha acids, amino acids, and organic acids depending on the detector used. The applicable detectors are RID (refractive index detector), PDA (photodiode array detector), RF (fluorescence

detector), and ECD (electroconductive detector). The Nexera X2 UHPLC can operate with one to four detectors and upgraded in the field as needs expand.

\* Note: typical laboratory equipment, such as chemicals, glassware, pipettes, shaker, centrifuges, etc., is not included.



# High-end Analysis for the Brewing Lab

This package enables you to determine water characteristics, mycotoxins, metabolomics, amino acids, inorganic water contaminants, carbon dioxide, organic water contamination, and tensile strength of beer can tabs.



Shimadzu LC-20AT Ion Chromatography System for water characteristics



Shimadzu LCMS-8050 for mycotoxins, metabolomics and amino acids



Shimadzu AA-7000, ICPE-9820, or ICP-MS for inorganic water contamination



Shimadzu TOC-L Total Organic Carbon Analyzer for carbon dioxide determination and organic water contamination



Shimadzu EZ-X tensile testing machine for testing beer tab strength

\* Note: typical laboratory equipment such as chemicals, glassware, pipettes, shaker, centrifuges, etc. are not included.



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Shimadzu Supports Beer Analysis with Analytical Techniques.

Shimadzu's Total Support for Beer Analysis

Analytical and Testing Instruments for Beer



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