Brewing QC Applications Using Headspace Sampling-Gas Chromatography

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Introduction

Headspace sampling coupled with gas chromatography (HS-GC) is a widely used technique for the analysis of beer throughout the world. HS-GC is typically used for quality control (QC), to identify problems or changes occurring in the brewing or fermentation process that affect the taste or quality of the final product.

Four of the major HS-GC analyses that are typically performed at breweries are described here (Table 1). The first, and most important, is monitoring for vicinal diketones (VDK) in the beer, which include 2,3-butanedione (diacetyl) and 2,3-pentanedione. VDK are considered extremely important since they are known to affect the taste of the beer. These components produce a butter-like flavor and are also considered non-beneficial at high levels. Many heavier beers, such as European beers, have VDK at higher levels than the lighter beers typically produced in the U.S. and they still maintain good flavor. VDK concentrations typically range from 1-50 ppb in lighter beers, but they can reach several hundred ppb in darker beers.

A second common HS-GC analysis performed in the brewing process is monitoring acetaldehyde. Acetaldehyde is reduced to ethanol by yeast during secondary fermentation, but oxidation of the finished beer may reverse this process, converting ethanol back to acetaldehyde. Acetaldehyde can also be a product of bacterial spoilage caused by Zymomonas or Acetobacter. In addition, background levels of acetaldehyde can be tasted in beers that use beech-wood chips to drop the yeast before it can be reduced to ethanol. Acetaldehyde has the taste and aroma of fresh-cut green apples and has also been compared to grass, green leaves and latex paint. The typical levels of acetaldehyde that are monitored are 1-20 ppm.

A third HS-GC analysis typically performed on beer is monitoring of trihalomethanes. These can be harmful and are usually introduced into the beer through the municipal water supply. Municipal water is often treated with chlorine, resulting in a variety of chlorinated hydrocarbon disinfection byproducts. The QC check for trihalomethanes is typically performed on incoming water, but not always on the large numbers of samples taken from the finished product. Chloroform is usually the most prominent trihalomethane component identified in this analysis.

The fourth HS-GC test commonly performed is for the identification of sulfur compounds in beer. Dimethyl sulfide (DMS), sulfur dioxide (SO_2), and hydrogen sulfide (HS) are monitored by some brewers. DMS has the taste and aroma of sweet corn. This comes either from the malt, as a result of the short or weak boil of the

wort, slow wort chilling or bacterial infection. Hydrogen sulfide is an indicator of the performance characteristics of the yeast, since some yeasts can produce significantly different levels of hydrogen sulfide. Sulfur dioxide is often encountered due to its presence as a preservative. When present in beer at low quantities, these sulfur components can be considered acceptable, but above very low ppb levels, they give off an unpleasant taste and smell (e.g., rotten eggs).

Although these four QC tests are usually performed individually, some breweries will combine two tests in the interest of time and sample throughput. For example, the test for VDK may also be used to identify the presence of chloroform, and the test for acetaldehyde may also be used to identify sulfur compounds.

Table 1. Four Typical HS-GC Analyses Performed in the Brewing Process.

1	Vicinal diketones (VDK)	2,3-Butanedione (diacetyl) 2,3-Pentanedione
2	Acetaldehyde	Acetaldehyde
3	Trihalomethanes	Dibromochloromethane Bromoform Chloroform Dichlorobromomethane
4	Sulfur	DMS (dimethyl sulfide) Sulfur dioxide Hydrogen sulfide

Experimental

All analyses were performed using a PerkinElmer® TurboMatrix™ automated headspace sampler (TurboMatrix HS-40 and HS-110) and a Clarus® 500 gas chromatograph (Figure 1). The Clarus 500 GC was configured with both a flame ionization detector (FID) and an electron capture detector (ECD).

Beer samples require degassing prior to headspace analysis. Full degassing of beer is important to prevent dissolved carbon dioxide (CO_2) from influencing vial pressure during the headspace heating process and to minimize GC baseline disturbances from CO_2 eluting during chromatography. Repeated shaking in an oversized container and allowing the foam to settle is one way to degas the samples, but filtration or sonication is easier and more efficient.

Samples were prepared by transferring the beer to a wide mouth beaker and sonicating them briefly (only 5-15 seconds is required). Using a wide-mouth beaker that is at least 10 times the volume of the beer measured is recommended. (Note: sonicating the beer directly in the bottle will cause an instantaneous geyser of beer foam to elevate 10-24 inches in height!) After degassing, 5-10 mL of beer sample was placed into a headspace vial (PerkinElmer part number B0104236) and sealed with PTFE/butyl rubber septa (PerkinElmer part number B0159356).

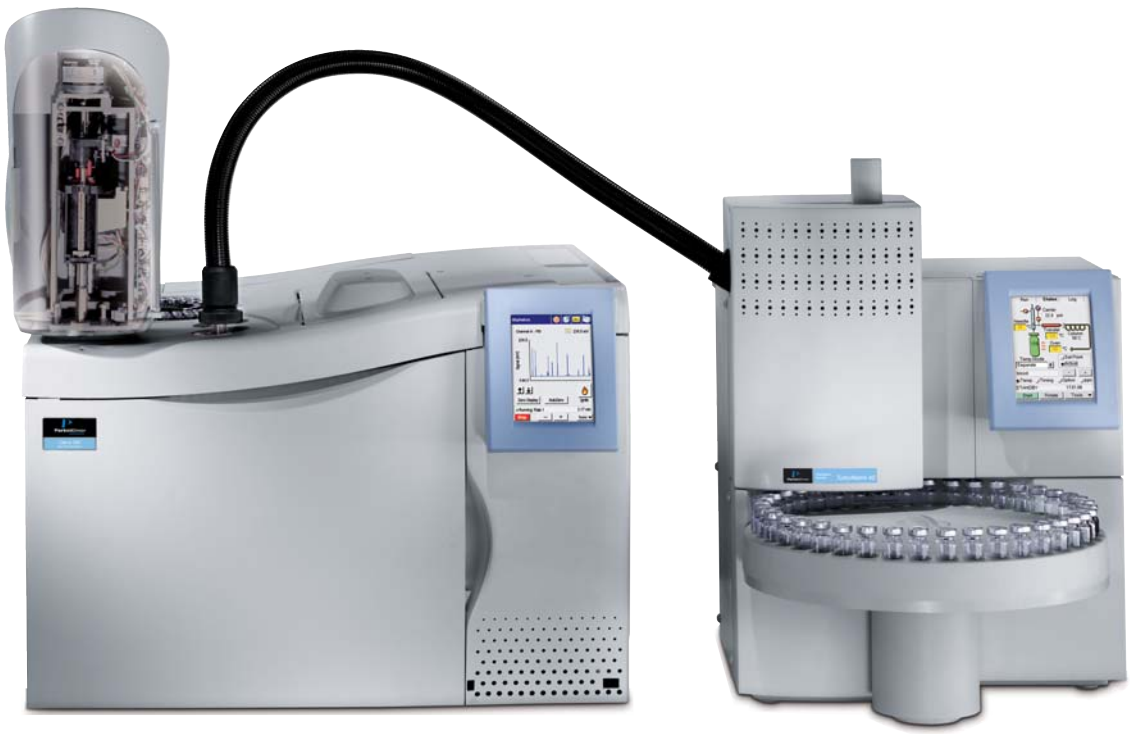

Figure 1. TurboMatrix automated headspace sampler (right) with the Clarus 500 gas chromatograph (left).

Results

Experiment 1 – Vicinal diketones: 2,3-butanedione (diacetyl) and 2,3-pentanedione

The desired 1-50 ppb detection limits are achieved by using an electron capture detector (ECD). The column used for the vicinal diketones analysis is an Elite-5, 60 meter x 0.53 mm x 1.5 µm (PerkinElmer part number N9316103). The HS and GC conditions required for the analysis are listed in Tables 2 and 3. A typical chromatogram showing the presence of VDK is displayed in Figure 2. Note: some beer methods use a manual headspace technique[1], requiring attended analysis and yielding less reproducible results than the automated system demonstrated here.

Table 2. HS Conditions.

Sample Temperature:	60 °C
Needle Temperature:	80 °C
Transfer Line Temperature:	100 °C
Equilibration Time:	15 min
Pressurization Time:	1.0 min
Injection Time:	0.1 min
Withdrawal Time:	0.0 min
Carrier Pressure:	35 psi

Table 3. GC with ECD Conditions.

Initial Temperature:	45 °C
Hold Time 1:	1.3 min
Rate 1:	40 °C/min
Final Temperature:	150 °C
Hold Time 2:	0.6 min
Injector Temperature:	100 °C
Liner:	Zero Dilution
Split:	25 mL/min
ECD:	150 °C
ECD Attenuation:	1
Makeup Gas (Argon/Methane):	30 mL/min

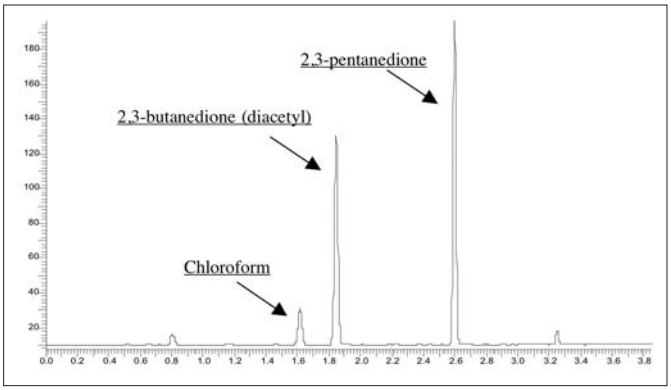

Figure 2. Vicinal diketones determination at 10-ppb concentration (ECD).

Experiment 2 – Acetaldehyde

The detection limits required for acetaldehyde determination (1-20 ppm) are achieved using a flame ionization detector (FID). The column used for the acetaldehyde experiment is an Elite BAC-1, 30 meter x 0.32 mm x 1.8 µm (PerkinElmer part number N9316579). The conditions required for the headspace sampler and gas chromatograph are listed in Tables 4 and 5. A chromatogram showing the presence of acetaldehyde along with 2-propanol, which is used as the internal standard in this analysis, is displayed in Figure 3. Note that dimethylsulfide is also identified in the chromatogram, confirming the presence of this sulfur-containing compound.

Table 4. HS Conditions.

Sample Temperature:	60 °C
Needle Temperature:	80 °C
Transfer Line Temperature:	100 °C
Equilibration Time:	15 min
Pressurization Time:	1.0 min
Injection Time:	0.1 min
Withdrawal Time:	0.0 min
Carrier Pressure:	35 psi

Table 5. GC with FID Conditions.

Initial Temperature:	45 °C
Hold Time 1:	1.3 min
Rate 1:	40 °C/min
Final Temperature:	150 °C
Hold Time 2:	0.6 min
Injector Temperature:	100 °C
Liner:	Zero Dilution
Split:	25 mL/min
FID:	150 °C

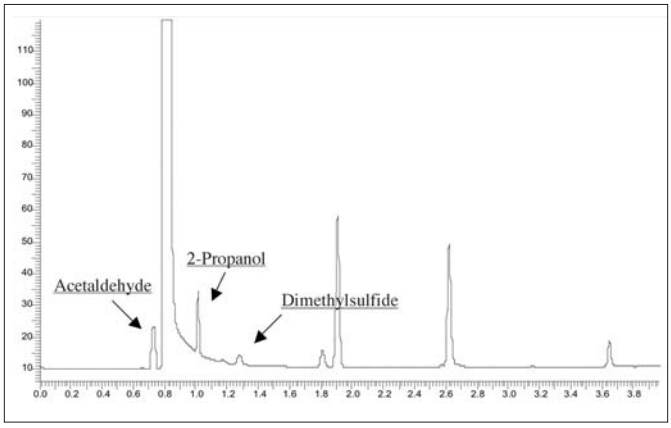

Figure 3. Acetaldeyde determination at 10-ppm concentration (FID).

Experiment 3 – Trihalomethanes (THMs)

The low-ppb detection limits necessary for trihalomethane analysis are achieved using an electron capture detector (ECD). THMs are introduced into the process with the water used to make the beer. The THM test can be run on incoming water, processed water and also the beer itself. To measure all four trihalomethanes (chloroform, dichlorobromomethane, bromoform and dibromochloromethane), a temperature-programmed GC analysis is required. Consequently, this is not considered a high-throughput application. Many brewery QC labs will determine the presence of chloroform (typically the most common THM) during VDK analyses of the finished product, as depicted in Figure 2. However, other labs will perform a separate analysis to determine total THM content.

Experiment 4 – Sulfur Compounds

Low-ppm detection limits for sulfur-compound analysis can be achieved using a flame ionization detector (FID). Typically, dimethylsulfide is also quantified on the FID during the acetaldehyde determination, so the column and conditions used are those listed under Experiment 2, and Figure 3 displays an example chromatogram. If ppb detection limits are required, a sulfur detector such as a Chemiluminescence or another sulfur-specific detector would be required with your PerkinElmer GC.

Conclusions

The PerkinElmer HS-GC system has the capabilities needed to perform the vital QC checks required throughout the beer-making process. Undesirable components introduced into or created by the brewing process can be sampled, separated, identified and quantified using flame ionization or electron capture detection at a ppm or ppb level, respectively. In addition, the integrated system described here provides automated headspace analysis, yielding more reproducible results that can be acquired with unattended operation.

The four experiments described here can be performed separately, but some QC labs will perform two of the experiments simultaneously due to throughput considerations. For example, sulfur compounds (typically DMS) will be determined during the acetaldehyde analysis and trihalomethanes (typically chloroform) will be determined during the VDK analysis. If these screening tests indicate that the targeted components exist at undesirable levels, more specific analyses will typically be performed as part of a follow-up procedure.

It is possible to perform all four analyses simultaneously on the same HS-GC system. This entails splitting the GC column effluent between the FID and ECD detectors, and choosing a GC column and conditions that will separate all the components. The Elite-5 column – 60 meter x 0.53 mm x 1.5 µm (N9316103) – has been successfully used to accomplish this. However, both the oven-temperature ramp and the overall run time have to be increased to successfully separate all the components of interest, so the overall throughput of this analysis method is low.

References

1. Methods of Analysis, The American Society of Brewing Chemists, Eighth Revised Edition St Paul, MN, 1992 (some sections revised 1996).

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