Analysis of Sulfur Compounds in Commercial Beers

Application Note 228-304

Atomic emission Detection

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Abstract
A system for the selective determination of volatile sulfur compounds in beer is described. Sulfur chromatograms for several beers are compared. The relative changes in volatiles during aging of one beer at 80°C is shown. Examples of the use of spectra for confirmation of the presence of sulfur are given.

Introduction
This paper investigates the use of the HP 5890 Series II gas chromatograph (GC) with the HP 5921A atomic emission detector (AED) and with the highly inert HP 5971A headspace sampler for analysis of volatile sulfur compounds found in commercial beer samples.

Traces of volatile sulfur compounds are of interest in many industries. In food and beverages, these compounds are important components of both desirable flavors and off-flavors. The sulfur compounds are often noticeable even at sub part per million levels.

Headspace is a powerful technique for this type of analysis because volatile components of the sample can be analyzed at low concentrations while protecting the chromatograph from nonvolatile sample components (e.g., sugar, protein).

Headspace samplers have until recently been limited by the surface activity of the sample path. At trace levels, many sulfur compounds can tail or even be irreversibly absorbed. Recently a new headspace instrument, the HP 7694, has become available with its sample path entirely composed of a new, highly inert material, Silcosteel™. For some compounds, the surface of this material is even more inert than deactivated fused silica.

Detection of ultra traces of sulfur-containing compounds can be performed with several different detectors. The AED used here has sensitivity at least as good as competing techniques, a very good tolerance for hydrocarbon background (good selectivity), and constancy of elemental response factors between compounds. The AED also allows the presence of sulfur in compounds to be confirmed by spectral identification, even at low ppb levels.

In the case of sub ppm gas standards of volatile sulfur compounds, the AED allows quantitation of both sulfur and carbon and allows their ratios to be compared. In similar studies, this has allowed losses in the sample cylinder (which is not as inert as Silcosteel) to be confirmed.

Samples
Budweiser and Miller Lite were purchased in 12 oz bottles locally. The bottles were kept refrigerated at 1°C until analyzed. Various other brands of bottled beers were analyzed as well. These samples had been stored in a garage without refrigeration for approximately 18 months.

Sample Preparation
Headspace vials (20 mL) were flushed with a nitrogen flow of a few liters per minute. Immediately
after removing the bottled beer from the refrigerator, disposable 10 mL pipets were rinsed twice with the beer to be analyzed. Approximately 10 mL of beer, not counting a small amount of foam that formed in the pipes, was delivered to the headspace vial, which was then capped.

A group of samples (from three to twelve) was then loaded into the sample cassette of the headspace autosampler. The first sample in a group was then loaded automatically into the oven within 5 minutes. The remaining samples were loaded into the oven "N" minutes in advance of the injections. The injections were repeated every 55-65 minutes, typically. The equilibration time "N" was determined as part of the experiment.

**Typical Chromatograms**

Figure 1a shows some typical carbon and nitrogen chromatograms, showing CO₂ at 3-4 minutes. There is a large baseline disturbance caused by column "bleed" after 20 minutes. Ethanol elutes at 23.3 minutes.

Water elutes at about 12 minutes. It was decided to vent water (see "AED Conditions" in table 1). However, the step was probably not necessary.

The solvent vent time (11.3 to 14 minutes) shows as a positive response on both the carbon and nitrogen channels. This is deliberate. During wavelength calibration (performed automatically from time to time in the few minutes before an injection), solvent venting is turned on, and the level of carbon and nitrogen visible in figure 1a is used to produce emission lines as wavelength standards.

**Peak Identification**

Figure 1b shows a sulfur chromatogram recorded simultaneously with the C and N chromatograms of figure 1a. Four sulfur-containing peaks have been identified as labeled. Three other peaks that probably contain sulfur have not been identified and are labeled with an X, Y, and Z.

Peaks were identified initially by matching the retention times with similar runs. Data were available from two polymer analyses of sulfur impurities and confirmed the retention time of H₂S, COS, and methyl mercaptan.

Data were also available from a beer headspace analysis using a similar porous polymer column, GS-Q. This allowed the identification of dimethyl sulfate (DMS) as well as some unidentified sulfur compounds; one of these matches an unknown found here.

The presence of sulfur was confirmed in the four larger sulfur-containing peaks by examining the spectra, as described in a later section.

Other peaks (N₂ + O₂, CO₂, ethanol, and water) were identified by the expected retention time, by consulting...
tables of relative retention times for the somewhat similar GS-Q column, and by the presence and absence of various elements. Some of the peaks of interest are listed in table 2.

**Quantitation**

No standards were available for this work. However, similar studies for volatile sulfur impurities in monomers have been performed recently by the AED with the gas sampling valve introduction. The same column and temperature program was used as in this work. A gas standard was used that included several of the compounds (H\textsubscript{2}S and COS) used here.

Low ppb (volume/volume) concentrations were injected with a 1.6 mL loop. The response factors were scaled by 1.6 to give equivalent response factors to the 1.0 mL sampling loop used here. Mono-sulfur compounds, such as COS, had response factors of about 4 area counts per ppb (v/v) for a 1 mL loop.

In earlier work on 10 sulfur compounds in whiskey, the response factor (per picogram of elemental sulfur) was found to be constant to about 10% within the same chromatogram. A larger uncertainty, perhaps + 50%, should be used here because the exact flows are not likely to be the same as in the earlier work, and the AED's response varies with both the makeup gas and the added reagent gases, such as oxygen.

This response factor can be used to estimate the concentration of sulfur compounds in the headspace over the beer sample. The concentration in the liquid was not estimated because there
were no standards available for this study.

Using the 4 counts/ppb response factor, the labeled peaks in figure 1 correspond to the following concentrations in the headspace: H$_2$S, 2.2 ppm (v/v); COS, 0.17 ppm; methyl mercaptan, 0.33 ppm; and dimethyl sulfide, 5.9 ppm. Based upon the scatter in the data for COS (see table 3, readings 4 through 8), the method appears to be precise to about 50-75 area counts, or about + 16 ppb. No attempt was made to improve this number or even measure it accurately. The monomer study mentioned above found % RSD (percent relative standard deviation) precision values of 2.5% for the analysis of 400 ppb of H$_2$S and 500 ppb of COS. Here, better data might be found if replicate analyses were made under identical conditions. Sensitivity could be improved by using a 3 mL loop and possibly by optimizing the makeup and reagent flows into the detector.

**Test for Reaction in the Vial**

The question arose as to what extent the compounds of interest are formed or degraded during equilibration at high temperatures prior to headspace injection. This was investigated easily using the automation capability of the headspace sampler.

A series of 12 samples were made, all from the same bottle of Samuel Adams beer. The first was equilibrated at 15 minutes at 80°C. The next at 30 minutes, up to the twelfth sample, which was equilibrated for 180 minutes. An injection was made from the first sample within 20 minutes of opening the beer bottle. The last sample was analyzed about 21 hours later. Prior to entering the equilibration oven, the samples were stored in the sample cassette at room temperature. The conditions are given in table 1.

The area results for the sulfur peaks are shown in table 3. The DMS peak at 26.6 minutes shows a response constant to within 5% over all 12 determinations. This variation is thought to be due mostly to pipeting errors because no great care was used, and the presence of foam complicated measurement. DMS shows no significant change in response with equilibration time.

The H$_2$S peak at 10.6 minutes shows an increase in response as equilibration time is changed from 15 to 30 and perhaps 45 minutes. Its highest response is for the fourth sample (60 minute equilibration). As the equilibration time is gradually increased by a further 2 hours, the peak response drops by 10 to 15 percent. Figure 2a shows the trends for the two largest peaks.

For the four medium-level components, COS, methyl mercaptan, and the peaks at 27 and 28 minutes, there is a low or nondetected response at the 15 minute equilibration time. There is a strong increase in response from the first to the third or fourth analyses (15 to 45 minutes, 60 minutes equilibration time). For most of the remainder of the test, the responses continue to increase but at a slower rate. Figure 2b shows the trends for the four compounds.

Figure 3 shows the sulfur chromatograms for the second sample (equilibrium time of 30 minutes) and the twelfth sample (equilibrium time of 180 minutes).

The smallest peak in table 3, at 20.4 minutes, is not plotted. Unlike the peaks in figure 2a, it does not seem to show a systematic increase with equilibrium time. In the runs in figure 3 and several others, a possible sulfur peak between 33 and 34 minutes is also visible near the baseline noise. However, it is noted that both the 20.3 minute and the 33 to 34 minute peaks seem to be simultaneous with a carbon-containing peak in figure 1a.

### Table 3. Area Results from the Sequence: SBR7A01.A.D to -A12.A.D Equilibration Time Varies 16, 30, ...180 Minutes

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound</th>
<th>Run</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.57</td>
<td>H$_2$S</td>
<td>3391.3</td>
<td>204.43</td>
<td>—</td>
<td>444.86</td>
<td>26246</td>
<td>412.78</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14.27</td>
<td>COS</td>
<td>8695.5</td>
<td>616.41</td>
<td>73.76</td>
<td>825.08</td>
<td>25736</td>
<td>252.52</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20.41</td>
<td>CH$_3$SH</td>
<td>9090.8</td>
<td>751.04</td>
<td>63.82</td>
<td>894.22</td>
<td>24866</td>
<td>560.48</td>
<td>1.56.27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>21.80</td>
<td>DMS</td>
<td>10389</td>
<td>977.39</td>
<td>83.88</td>
<td>980.69</td>
<td>24990</td>
<td>786.68</td>
<td>198.79</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>26.59</td>
<td>COS</td>
<td>10019</td>
<td>902.69</td>
<td>70.02</td>
<td>969.55</td>
<td>24457</td>
<td>943.84</td>
<td>111.21</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>27.319</td>
<td>COS</td>
<td>9848.0</td>
<td>843.15</td>
<td>65.17</td>
<td>1065.40</td>
<td>23923</td>
<td>778.51</td>
<td>125.85</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>28.1-3</td>
<td>X</td>
<td>10362</td>
<td>981.07</td>
<td>86.46</td>
<td>1139.70</td>
<td>26180</td>
<td>879.88</td>
<td>251.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The conditions are given in table 1.
Comparing Various Beers

Three brands of beer and a distilled water control were analyzed. Conditions are listed in table 4.

Figure 4 shows the sulfur run for the second sample of beer, Miller Lite at a high sensitivity, the DMS peak at 26 minutes is about 10 times offscale. The common sulfur compounds, H$_2$S, COS, CH$_3$SH, and DMS, are labeled. The 20, 27 and 28 minute unknown peaks are visible. The apparent sulfur peak at 23.5 minutes is due to ethanol because of its large relative concentration, but the possibility of a coeluting sulfur peak cannot be ruled out.

Figure 4 also shows a sulfur chromatogram for the distilled water blank at the same sensitivity. This sample was run just after the beer sample at the top of figure 4. There is little carryover from previous samples on the sulfur channel.

Table 5 shows the results for the three beers analyzed. No areas are listed for some of the unknown sulfur peaks—more sensitive integrator settings were used previously in table 2 where these compounds were quantified.

Testing Lower Equilibration Temperatures

It was suggested that 80°C was too high to prevent reactions within the vials. A series of beer samples were, therefore, run at lower equilibrium temperatures. Conditions are shown in table 6.

In this series of measurements, a different temperature program was used. There were differences in data rate, injection time, and solvent vent time. Except for
equilibration time, these changes had only minor effects, if any, on the results.

During these runs, a survey was made for selenium compounds because in certain food products, selenium analogs of the volatile sulfur compounds have been found.

After the three-element analysis had been completed, it was decided to expand the survey to include the halogens. The next four elements were run using a separate sequence, rather than all seven elements in a single sequence. No selenium, chlorine, or bromine compounds were detected in any of the samples.

The sulfur runs for the three beers are shown in figure 5. The two major peaks, \( \text{H}_2\text{S} \) and DMS (at 10 and 30 minutes in this run), are much lower than in runs at an 80°C equilibration temperature. In these runs, COS is only barely visible at 13.5 minutes.

In the Kirin sample, the larger levels may be due to degradation during storage. Unfortunately, no fresher samples of this beer were analyzed. It is noted at the lower equilibration temperature, while there is lower response to sulfur compounds, there is also a large reduction in interferences from ethanol on the sulfur channel. However, there is still the same characteristic increase in baseline noise at the later retention times. This is due to the high level of interfering molecular spectra from the carbon-containing "bleed" near the sulfur emission lines. The software that provides high selectivity can null the response to this emission, but not the noise it causes. The area results are shown in table 7 for the three beers.
Spectral Confirmation

The following spectra were collected during the last chromatogram of the SBR7 series, which measured dependence on the thermal equilibration time. See figure 1 and table 3.

Figure 6 shows the characteristic three-line sulfur spectrum (180.7, 182.0, and 182.6 nm) during the elusion of H₂S. There are a few traces of hydrocarbon derived spectra.

In figure 7, a spectrum slightly earlier than the chromatographic peak has been subtracted from the H₂S spectrum so that the three sulfur lines are on a flat baseline.

In figure 8, the same background subtracted spectrum is shown in three dimensions. All three spectral lines are correlated during peak elusion. The absence of the characteristic hydrocarbon spectra in the vicinity of the sulfur triplet proves that no large organic compound was eluting at the same time.

Figure 9 shows the three-dimensional spectra during the DMS peak. There is a much higher level of hydrocarbon eluting at this retention time, so there are larger interferences from carbon-containing bands. Note that the hydrocarbon emission does not seem to be organized into discrete peaks; it is probably due to the "bleed" seen in the carbon chromatogram at this retention time.

Figure 10 shows the three-dimensional spectra during the much smaller COS peak. Because the sulfur emission is much smaller than the interfering bands, a more powerful technique to reject background, called

Table 5. Area Results from the Sequence: SBR6A O1A.D to -AO4A.D

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound: H₂S</td>
<td>5916.4</td>
<td>124.28</td>
<td>214.23</td>
<td>15215</td>
<td>212.71</td>
<td></td>
</tr>
<tr>
<td>Miller Lite</td>
<td>1406.1</td>
<td>87.31</td>
<td>119.47</td>
<td>8250.0</td>
<td>85.80</td>
<td></td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Samuel Adams</td>
<td>4049.7</td>
<td>370.7</td>
<td>50.63</td>
<td>545.66</td>
<td>24051</td>
<td>294.46</td>
</tr>
</tbody>
</table>

Table 6. Conditions for Test of Lower Equilibrium Temperature (conditions the same as table 1 unless noted)

<table>
<thead>
<tr>
<th>Samples</th>
<th>10 mL of bottled beer</th>
<th>Analyzed on September 8-9, 1993</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headspace</td>
<td>Equilibration time</td>
<td>35 min</td>
</tr>
<tr>
<td></td>
<td>Thermostating temperature</td>
<td>50°C</td>
</tr>
<tr>
<td></td>
<td>Loop/transfer temperature</td>
<td>55</td>
</tr>
<tr>
<td>GC Conditions</td>
<td>Temperature program</td>
<td>35°C for 2 min, 5°C/ min to 190°C hold for 10 min</td>
</tr>
<tr>
<td>AED Conditions</td>
<td>Solvent time</td>
<td>None used</td>
</tr>
<tr>
<td></td>
<td>Data rate</td>
<td>2.5 Hz</td>
</tr>
<tr>
<td></td>
<td>Elements recorded</td>
<td>C (uv), S, Se, C (visible), Cl, Br, H</td>
</tr>
</tbody>
</table>

Figure 5. Sulfur compounds in aged Kirin, Budweiser, and Miller Lite run at a lower equilibrium temperature (50°C). Conditions are given in table 6.

Table 7. Area Results from the Sequence SBR3

<table>
<thead>
<tr>
<th>Retention Time:</th>
<th>9.92</th>
<th>29.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound: H₂S</td>
<td>1690</td>
<td>8680</td>
</tr>
<tr>
<td>Miller Lite</td>
<td>290</td>
<td>4570</td>
</tr>
<tr>
<td>Kirin</td>
<td>770</td>
<td>12500</td>
</tr>
</tbody>
</table>
"background stripping," is used. Background stripping removes nearly all the hydrocarbon bands, which are much larger than the sulfur emission.

The slow tilt downward (to lower wavelengths and to later times) of the spectrum in figure 10 is due to a very broad emission band of O2, which is not corrected by background stripping. In C, N, and S runs, oxygen is added to the column effluent to prevent graphite formation in the AED discharge tube. Reagent gas flows are not listed in the tables of conditions because the flow is set automatically, once elements are selected for analysis. As the hydrocarbon emission increases, the O2 emission is reduced because a higher proportion of it is converted to CO.

Figure 11 shows a three-dimensional display of the methyl mercaptan peak. The interfering spectrum (O2 with a small amount of CO) increases rapidly with retention time. Even with back-ground stripping, the three sulfur peaks are still relatively hard to see.

The confirmation of the small "sulfur" peaks at 27.3 and 28.3 minutes was not successful. There are some indications, however, that sulfur emission is present, but the spectra is not persuasive.

Figure 12 shows possible sulfur emission at 27.3 minutes. The five offscale lines or bands in the background are from the DMS elusion at 26.58 minutes. From the left, the sulfur emission lines are the second, fourth, and fifth peaks. In the foreground, a "spine" is drawn over apparent maxima at 180.5, 182.0, and 182.5. These are possibly sulfur emission.

**Conclusions**

The combination of an inert headspace sampler with an atomic emission detector has proven very powerful for the selective determination of volatile sulfur components in beer. Element-specific chromatograms simplified locating potential sulfur-containing components, and evaluation of spectra helped to confirm its presence.

Beer equilibrated at high temperatures showed systematically varying amounts of sulfur compounds, indicating a complex degradation pathways.
Figure 8. Three-dimensional spectrum of H$_2$S. Background subtraction at 10.0 minutes.

Figure 9. Three-dimensional spectrum of DAMS. Background subtraction at 27.1 minutes.

Figure 10. Three-dimensional spectrum of COS. Background stripping at 14.6 minutes.

Figure 11. Three-dimensional spectrum of methyl mercaptan. Background stripping at 14.6 minutes.

Figure 12. Evidence of possible sulfur content in unknown eluting at 27.3 minutes.