Low-Level Analysis of Sulfur Compounds in Beer by Purge and Trap

Technical Overview

Introduction

Low levels of sulfur compounds in beer are known to have drastic effects on flavor and aroma. Levels as low as 0.1 ng/mL for compounds such as thiols can affect flavor and are indicators of variations in the brewing process. This makes it essential for the modern brewing industry to detect and monitor sulfur compounds in beer. In the past, the detection of such low levels of sulfur compounds was limited by the reactivity of the sulfur compounds with nickel tubing and stainless steel fittings within the analytical equipment. This made it difficult to recover analytes at low levels.

The quantitative analysis of low-level volatile sulfur compounds in beer is fully automated with the Tekmar AQUATek 70 and 3100 Sample Concentrator. An Agilent 6890 Gas Chromatograph (GC) with an Agilent Model 355 Sulfur Chemiluminescence Detector (SCD) were used for this analysis. The sample pathway of the 3100 Sample Concentrator is completely Silcosteel, creating the perfect pathway for sulfur compounds. Low-level sulfur compounds do not adsorb or decompose on the inert Silcosteel surface. This surface is composed of a layer of inert fused silica coating the inside of the sample pathway.

The results show the detection limits achieved for sulfur compounds found in beer and the repeatability of the results. A comparison of two gas chromatograph column types used for this study, an Agilent GS-GasPro and an Agilent DB-1 are also shown.

Experimental

Each beer sample was poured into an amber 40-mL vial. To reduce foaming of the beer, 0.1 to 1 mL of Dow Chemical Defoamer 1520 (diluted 1:5 with water) was added to the beer samples and blanks. To bind metals present in the beer, 0.15 grams of ethylenediaminetetraacetic acid, disodium salt dihydrate, 99+% (EDTA) was added to each vial. The EDTA showed no measurable improvement in the response of the analytes; however, it was used to prolong the useful life of the ceramic tubes inside the detector. The samples were placed in the 70-position vial tray of the Tekmar AQUATek 70. The samples were spiked automatically by the AQUATek 70 with 2 µL of the internal standard, isopropyl sulfide (200,000 ng/mL) and were transferred to the 3100 Sample Concentrator. The 20-mL sample was then purged in a fritless 25-mL sparger (p/n 14-4826-024). After the volatiles are purged onto the trap they are desorbed to the GC where they are separated and analyzed by the SCD.

The analytical instruments were calibrated using standards that were prepared in water with an adjusted pH and ethanol content that mimic beer. Buffer tablets were used to prepare the water to a pH of 4. Vodka was used to adjust the ethanol content of the water to 4%. The parameters used are shown below in Tables 1 through 5.

Table 1. Tekmar 3100 Parameters

3100 Sample Concentrator	
Purge time:	7 min
Desorb temperature:	225 ºC
Desorb time:	2 min
Bake temperature:	225 ºC
Bake time:	10 min
Line and valve temperature:	150 ºC
Trap:	Glass-lined Tenax (p/n 14-4045-303)

Table 2. AQUATex 70 Parameters

AQUATek 70	
Sample volume:	20 mL
Fill IS:	0.04 min
Transfer IS:	0.75
Rinse lines:	0.75 min
Bake rinse:	0.75 min
Rinse cycles:	1
Fill IS:	On

Table 3. SCD Parameters

Agilent Model 355 SCD	
Pressure controller:	150–275 torr SCD: 5–10 torr
Burner temperature:	800 ºC
Hydrogen flow rate:	100 mL/min
Air flow rate:	40 mL/min
Background signal:	0.3–0.8 mV

Table 4. GC Parameters for the DB-1 Column

GC Parameters	
Agilent 6890 Gas Chromatograph	
Column:	Agilent, DB-1, 0.53 mm id, 30 m length, 5 µ film thickness
Inlet:	Bypassed with a direct connection between transfer line and column using a zero dead volume union (p/n 14-2069-016). An external pressure regulator was used to maintain a head pressure of 5 psi.
Oven:	35 ºC hold for 5 min 1 ºC/min to 50 ºC 50 ºC hold for 0 min 15 ºC/min to 200 ºC 200 ºC hold for 0 min

Table 5. GC Parameters for the GS-GasPro Column

GC Parameters	
Agilent 6890 Gas Chromatograph	
Column:	Agilent, GS-GasPro, 0.32 mm id, 60 m length
Inlet:	Bypassed with a direct connection between transfer line and column using a zero dead volume union (p/n 14-2069-016). An external pressure regulator was used to maintain a head pressure of 20 psi.
Oven:	50 ºC hold for 3 min 15 ºC/min to 260 ºC 260 ºC hold for 25 min

Results and Discussion

Table 6 shows the compounds that were calibrated for this study and their quantification levels. These levels are based on the flavor and odor threshold of the compounds. Each compound was calibrated using a linear calibration of 5 points that bracketed the quantification level. However, the analytical response of this method makes it possible to detect even lower concentrations. Table 7 shows the repeatability of English pale ale analyzed by this study. Table 8 shows the percent recovery of 4-ng/mL concentration spiked into the beer. Figure 1 shows a chromatogram of a European pilsner beer run on a DB-1 column. Figure 2 shows the same beer run on a GS-GasPro column.

Table 6. Compound List with Quantification Level and Calibration Data Using a DB-1 Column

Compound	Quantification level (QL) (ng / mL)	%RSD at QL (n = 7)	Range of calibration curve (ng / mL)	Correlation coefficient of calibration (r^2)
Ethanethiol	0.1	5	0.1–4	0.994
Dimethyl sulfide	10	3	0.24–12	0.999
Carbon disulfide	15	3	4–40	0.967
Ethylene sulfide	0.1	2	0.1–4	0.990
Propanethiol	0.1	9	0.1–4	0.974
Methyl thioacetate	10	10	4–40	0.998
Ethyl thioacetate	0.5	4	0.1–4	0.998
Dimethyl disulfide	0.5	3	0.24–12	0.999
Dimethyl trisulfide	0.05	4	0.016–4	0.998
Ethyl methyl sulfide	0.5	3	0.24–12	0.999
Diethyl sulfide	0.5	4	0.1–4	0.985

Table 7. Repeatability Study Results Using a GS-GasPro Column

Compound	Average concentration (ng / mL)	%RSD (n = 9)
Dimethyl sulfide	8.1	10.2
Diethyl sulfide	0.94	1.1
Ethyl methyl sulfide and dimethyl disulfide	0.78	17.7
Methyl thioacetate	0.98	13.0

Table 8. % Recovery Study Results Using a GS-GasPro Column.

Compound	% recovery study of 4 ng / mL
Dimethyl sulfide	77
Diethyl sulfide	75
Ethyl methyl sulfide and dimethyl disulfide	89
Methyl thioacetate	84

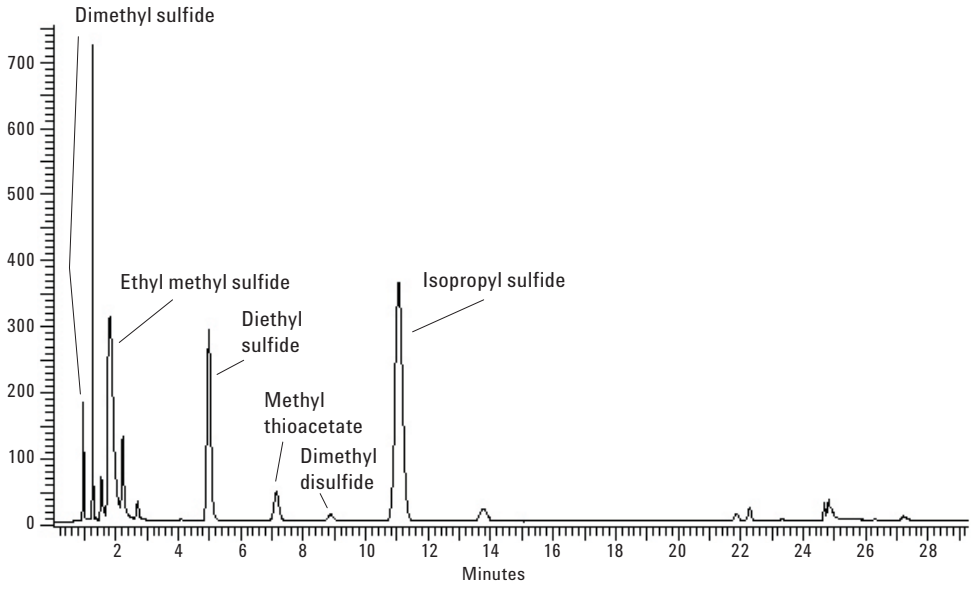

Figure 1. DB-1 chromatogram of a European pilsner beer.

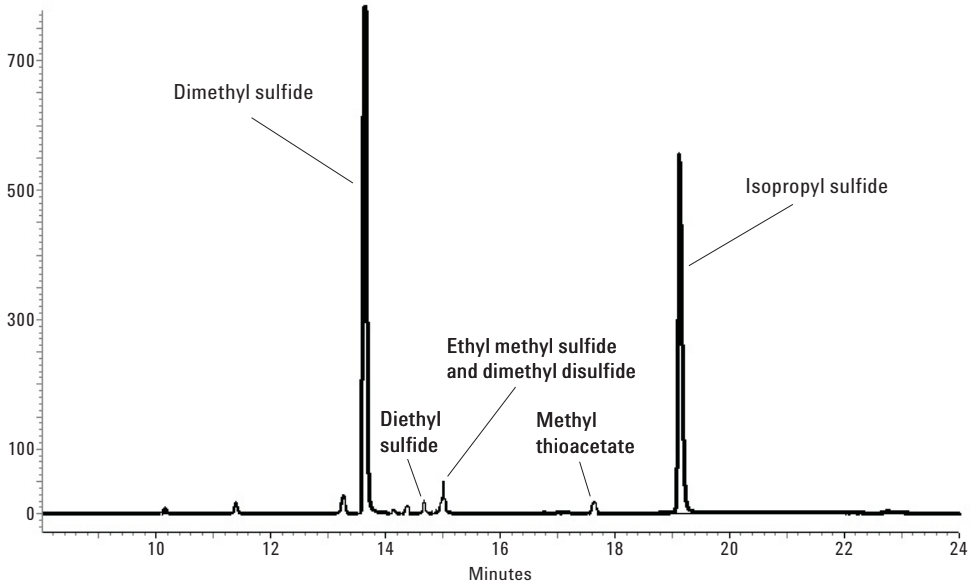

Figure 2. GS-GasPro chromatogram of a European pilsner beer.

Conclusions

The results of this study show that sulfur compounds in beer are easily quantified, at or below the flavor and odor threshold, with a high level of precision. The results displayed in Table 6 for the percent relative standard deviation (%RSD) for seven replicates at the quantification level show that the precision of the method is good. The correlation coefficients of the calibration curve demonstrate the linearity of the calibration. Table 7 shows the repeatability of this method as it directly applies to analyzing a European pilsner. The %RSD for nine replicates of beer confirms the method is very precise. The % recovery study shows that the accuracy for this method is good.

The chromatograms in Figure 1 and Figure 2 show the results of running a beer with a DB-1 column compared to a GS-GasPro column. The DB-1 column offers the advantage of better separation of peaks. The GS-GasPro column had the advantage of better peak shape and less bleed.

The method used in this study allows brewers to detect and quantify sulfur compounds that have an effect on beer quality. Low threshold levels of sulfur compounds can be analyzed with a completely automated system leading to good precision and accuracy.

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