Rapid Quantification of Flavor-Active Sulfur Compounds in Beer

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ABSTRACT

Sulfury notes that did not correlate with measured dimethyl sulfide concentrations were identified during organoleptic panel assessments of beer. This initiated the development of a method by which these compounds could be detected, identified, and quantified. An in-bottle purge-and-trap sampling technique was successfully coupled to a capillary gas chromatograph with a sulfur chemiluminescence detector. Analyses of beer then generated sulfur compound profiles that correlated with the sulfury notes detected by the panelists. This method should be a valuable quality control tool for flavor-active sulfur compound profile measurement and for subsequent control of unwanted high concentrations of these compounds.

The advancement of gas chromatographic equipment has improved the scientist's ability to measure and correlate flavor-active compounds with sensory data (19). The influence of dimethyl sulfide (DMS) and 3-methyl-2-butene-1-thiol, two flavor-active sulfur compounds in beer, on beer quality has been widely studied and documented (1,2,4,6,8). However, non-DMS sulfury flavors often are present in some beers (12,17). The development of a rapid and accurate method to measure flavor-active sulfur compounds beyond DMS would contribute to the understanding of the role that these compounds play in beer taste and flavor.

Leppänen et al (7) reported an adsorption method for the concentration of volatile sulfur compounds from beer on Chromosorb 101 or 105 (Manville, Denver, CO). After thermal desorption, the compounds were separated directly on a chromatographic column using a dual flame photometric detector. A similar purge-and-trap desorb technique was used by Peppard (13), who used Porapak Q (Waters Associates, Milford, MA) as the adsorbent with a rapid heating and thermal focusing technique. More recently, Narziss and co-workers (9–11) reported results for in-process and packaged beer with a similar technique using a modified injection port.

As most reported methods involve a cumbersome, nonroutine sample preparation and introduction into the gas chromatograph (GC), our aim was to develop a technique that would reduce

sample preparation and automate sample introduction. These steps should improve reproducibility and sensitivity. Previous experience with automated purge-and-trap equipment for volatile analyses (3) prompted a modification of a similar unit for flavor-active sulfur compound analyses.

This article reports on the equipment modifications conducted to establish an automated beer sulfur compound profiling system. The use of this system to quantify sulfur compounds with precision and accuracy is described, as is the correlation of taste results with analytical data.

The need for a method to analyze volatile sulfur compounds while elminating artifact formation was important. The literature survey on the formation of volatile sulfur compounds revealed inconsistencies in the compounds found in beer and motivated the development of analytical tools that would limit artifact formation.

The exclusion of oxygen during sample preparation has been indicated as important by several authors in this field (14,16). An in-bottle sampling technique would assist in excluding atmospheric oxygen from sample preparation. This would also eliminate oxygen from the heat desorption step whenever a purge-and-trap method is used.

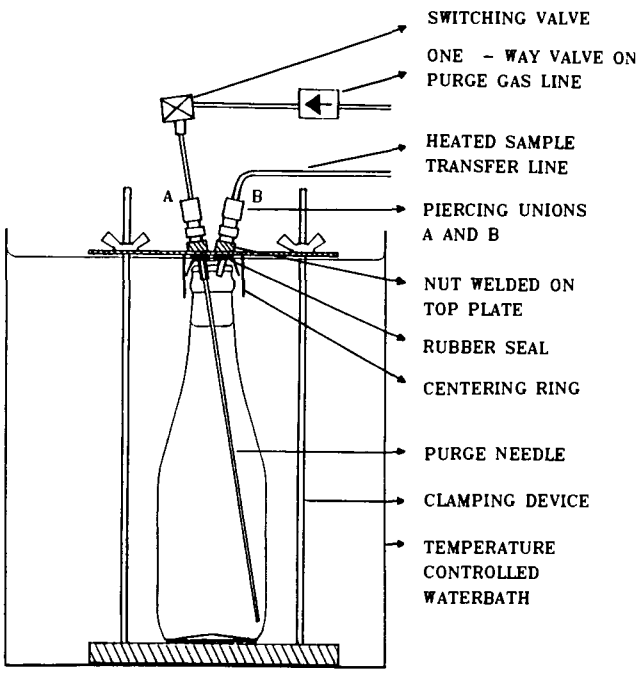

Fig. 1. In-bottle purging device developed for analysis of sulfur compounds in beer.

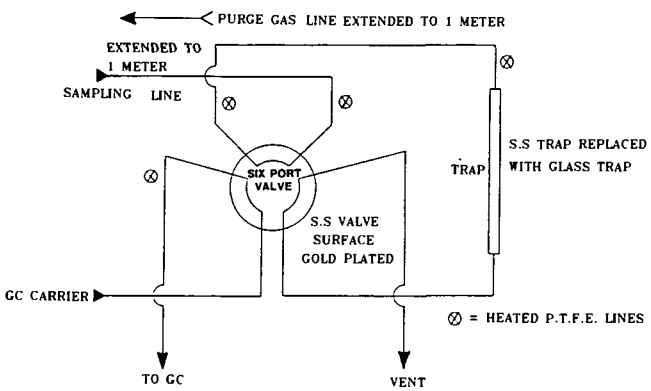

Fig. 2. Modifications of the Tekmar liquid sample concentrator to reduce metal surfaces in contact with the sample. GC = gas chromatograph. P.T.F.E. = polytetrafluoroethylene (Teflon).

EXPERIMENTAL

In-Bottle Purging Device

The in-bottle purging device (Fig. 1) consisted of a stand in which a bottle was firmly clamped and positioned under the piercing unions by means of a centering collar. The two piercing unions (A and B) were tightened down (through the threaded nuts, welded onto the top plate) to pierce the bottle crown. Leak-free connections were ensured by the rubber seals on the piercing tubes.

On the other end of each piercing union was a nut containing a GC septum through which the internal standard (IS) or the calibration mixture was introduced. A purging needle equipped with a switching valve was pushed through one of the piercing unions into the beer, supplying the purge gas. The in-line, one-way valve prevented flush-back of the sample into the purge gas line because of the pressure differential between the bottle and the purge gas.

The sample transfer line was equipped with a rigid polytetrafluoroethylene (Teflon) needle that was pushed through the septum of the second piercing union, from which the sample was transferred into the purge-and-trap concentrator.

Purge-and-Trap Sampler

Figure 2 shows a schematic diagram of the Tekmar LSC-2 (Tekmar Company, Cincinnati, OH) liquid sample concentrator, which was modified to minimize active metal surfaces. To achieve this, all metal transfer lines were replaced with Teflon lines. In

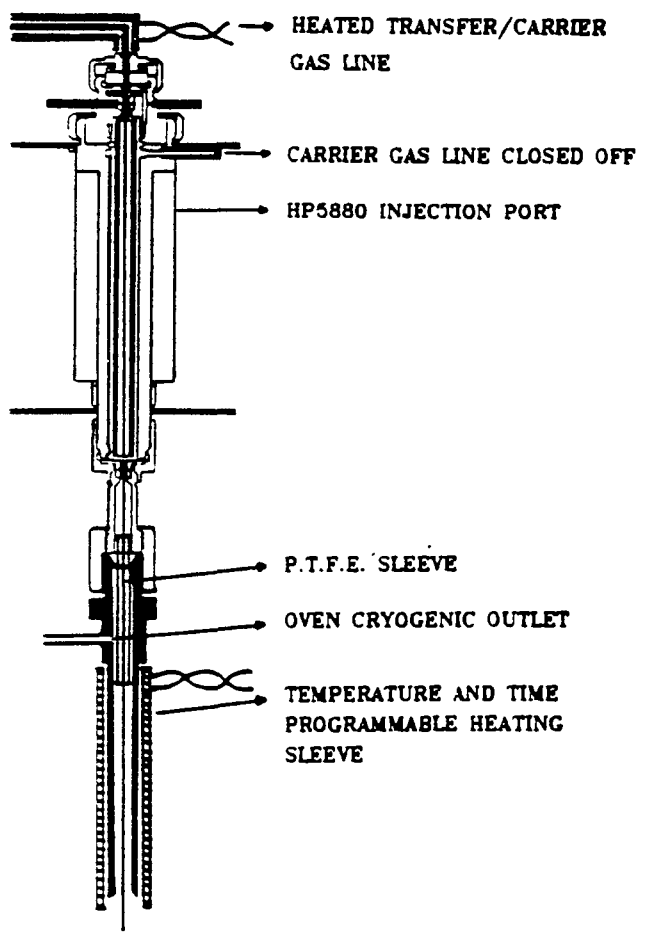

Fig. 3. Modifications to the Hewlett Packard (HP) 5880 injection port to facilitate sample cryofocusing and desorption. P.T.F.E. = polytetrafluoroethylene (Teflon).

addition, the six-port switching valve steel surface was gold plated, the stainless steel focusing trap was replaced with a glass trap, and the Teflon transfer line was extended into the focusing trap. To facilitate the in-bottle purging attachment, both the purge and sampling lines (on front panel of LSC-2) were extended to 1 m.

Modifications to the GC Injection Port

We modified the Hewlett Packard 5880 GC injection port (Hewlett Packard, Palo Alto, CA) (Fig. 3) to facilitate the attachment of the purge-and-trap concentrator and to improve chromatographic separation of the very volatile sulfur compounds.

The GC carrier gas was routed via the purge-and-trap concentrator through the transfer line so that the original carrier gas line could be closed off. The Teflon transfer line was pushed through the septum into the heated injection port. Sample splitting was achieved by inserting the column about 10 mm into the transfer line. A 6-mm injection port liner was attached to the 5880 GC injection port, and the liquid carbon dioxide cryogenic line was connected to the port liner. Once the oven was cooled to the initial temperature (20°C), the length of column passing through the liner was cooled to between −50 and −60°C. A focusing trap heater was fitted over the liner for heat desorption of the cryofocused sample. The heater then was time and temperature programmed through the GC terminal.

Optimization of Purge-and-Trap Parameters

For good integration and quantification, the purge gas flow rate was set at 40 ml/min, which was adequate to remove volatile material in the 2-min purge time. This flow rate also did not cause foaming of the beer at the start of the purge cycle. Because of the relatively high headspace pressure in the bottle promoted by temperature equilibration, the initial flow rate was substantially higher than the set rate. However, it stabilized within a short time to the set flow rate.

The maximum temperature at which the purge cycle could be started was set at 28°C. The trap temperature was usually about 26°C before the purge cycle commenced. The glass focusing trap was packed with 170 mg of Tenax TA (Alltech, Milan, Italy), which was preconditioned at 250°C for 60 min while purging with N_2. The purge time was set at 2 min. The desorb preheat temperature was set at 180°C to heat the focusing trap before the start of the sample transfer step.

The desorb time was set at 2 min to ensure complete transfer of all absorbed sample from the focusing trap. The trap bake-out temperature was kept at 200°C for 10 min to remove any absorbed compounds from the trap.

Continuous olfactory analysis of the outlet of the focusing trap confirmed that no breakthrough of the sulfur compounds occurred under the set conditions. No breakthrough of sulfur compounds could be detected, even when a calibration standard with relatively high concentrations was purged for 10 min.

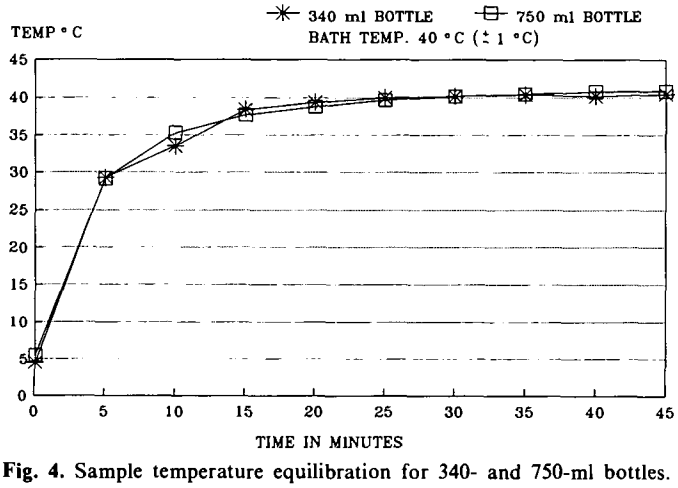

Fig. 4. Sample temperature equilibration for 340- and 750-ml bottles.

Sample Temperature Equilibration

A temperature of 40°C was selected for equilibration, based on the method developed for the headspace analysis of DMS (A. Darbyshire and K. Cosser, *personal communication*). This temperature provided sufficient volatile concentration for the analysis without excessive water and ethanol interference.

The equilibration time of 45 min was selected to coincide with the gas chromatographic analysis cycle. The relationship between temperature and time during the equilibration period of 340- and 750-ml beer bottles is shown in Figure 4. It takes about 25 min for the 340-ml bottle and 30 min for the 750-ml bottle to reach the equilibrium temperature. The 45 min allowed for equilibration should thus be sufficient to reach an equilibrium between the headspace and liquid in the bottles.

Sample Equilibration Preceding Analysis

Piercing union A was screwed through the bottle crown to introduce IS and calibration standards. Union B was screwed in after sample mixing was completed (Fig. 1). Then, 50 μl of diluted isopropyl sulfide (IPS) was injected before the onset of equilibration (sample bottle clamped in purging device) and inverted 20 times to ensure thorough mixing. The sample, firmly clamped in the purging device, was placed in a water bath at 40°C for 45 min. Just before the onset of the purge cycle, 30 μl of polypropylene glycol 2025 (GC grade) was introduced through one of the piercing unions.

Sample Collection and Transfer to GC

After crown piercing, the purge needle was introduced into the sample through piercing union A before the end of the equilibration period. The transfer line was pushed through union B at the end of the equilibration time. The purge cycle was commenced directly after the transfer line was inserted into the sample headspace. The switching valve on the purge gas line was opened only after the purge cycle commenced. At the end of the purge cycle, the switching valve was closed and the purge gas and sampling lines were reconnected to provide gas flow to bake out the focusing trap.

Analytical Conditions for Gas Chromatography

Gas chromatograph. An HP5880 GC was used for these studies. Initially, a 30-m × 0.53-mm DB-5 (1.5 μm phase) column was

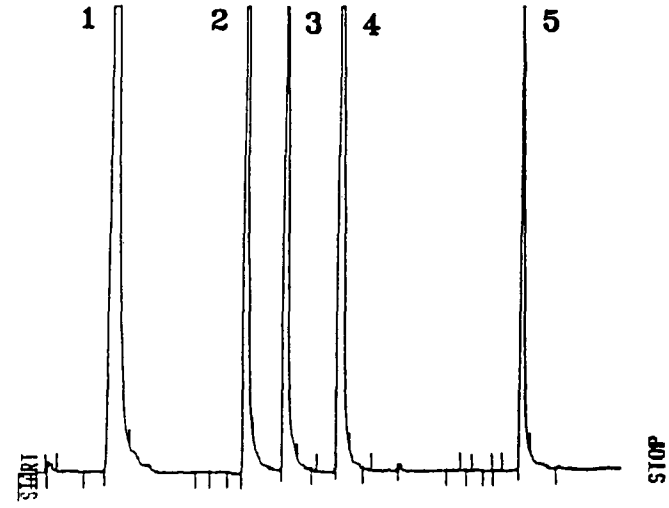

Fig. 5. Sulfur chemiluminescence detector chromatogram of calibration standards. 1 = Dimethyl sulfide, 2 = diethyl sulfide, 3 = dimethyl disulfide, 4 = isopropyl sulfide (internal standard), 5 = dimethyl trisulfide.

used (J & W Scientific, Cardova, CA), but when the column had to be replaced, a 30-m × 0.53-mm OV-1 (5 μm phase) column was selected (Ohio Valley Speciality Chemical, Marietta, OH). The flame-ionization detector (FID) flow rates were as follows. The air was set at 350 ml/min and the hydrogen at 360 ml/min for optimum sulfur chemiluminescence detector (SCD) sensitivity. Nitrogen was used as carrier gas and set at 12 ml/min, and nitrogen at 25 ml/min was used as makeup gas. The column split ratio was adjusted to give a column flow-split flow of 1:10 (12:120 ml/min). The injection port temperature was set at 150°C, and the FID temperature was set at 275°C.

Oven program. The initial temperature was kept at 20°C by cooling with liquid CO_2 for 2 min to correspond with the focusing trap desorb time. The column head desorb temperature was between −55° and −60°C, cooled with liquid CO_2 when the oven was cooled to 20°C.

First stage program. The oven was heated from 20 to 50°C at a rate of 5°C/min and held for 1 min.

Second stage program. The oven was heated from 50 to 180°C at a rate of 8°C/min and held for 10 min.

Analytical Conditions for the SCD

The SCD (Sievers Research, Boulder, CO) was initially stabilized by evacuating overnight in the standby mode with the FID flame on. Later, it was established that the SCD could be stabilized within 60 min without overnight evacuation provided that the FID flame was extinguished at least 30 min before the vacuum system was switched off. The transfer line was heated by means of a heating tape and rheostat at 80°C. Both the line and heater were insulated with rubber insulation hose. The sample probe was located 6 mm above the flame jet. The oxygen pressure was set at 0.6 kg/cm². A SCD sampling rate of 0.06 seconds was used, and the detector output was set at 100 mV.

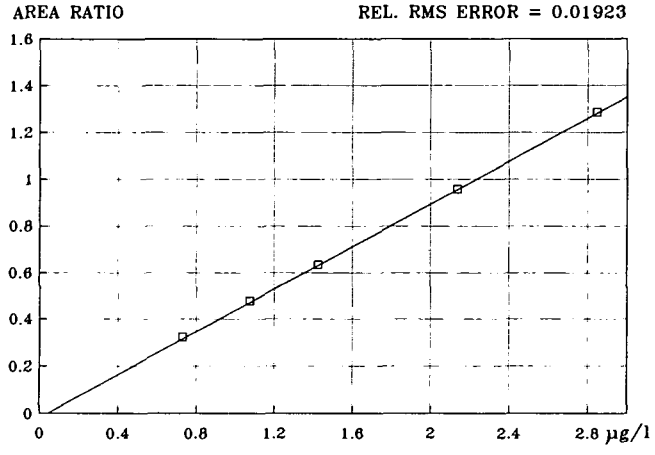

Fig. 6. Sulfur chemiluminescence detector calibration response curve for diethyl sulfide. Rel. RMS = relative root mean square error to a straight line fit.

TABLE I
Reproducibility of the Purge-and-Trap/Gas Chromatograph/Sulfur Chemiluminescence Detector System over 10 Consecutive Runs of Calibration Standards[a]

Run No.	Diethyl Sulfide	Dimethyl Disulfide	Dimethyl Trisulfide
1	2.11	0.76	2.31
2	2.13	0.77	2.11
3	2.15	0.80	2.13
4	2.09	0.77	2.03
5	2.04	0.75	2.34
6	2.13	0.78	2.39
7	2.18	0.81	2.27
8	2.27	0.83	2.06
9	2.08	0.77	2.32
10	2.10	0.78	2.65
Mean	2.13	0.78	2.26
SD	0.06	0.02	0.19
Percent relative SD	2.97	3.12	8.26

[a] Results reported in micrograms per liter.

Chemicals

The chemicals used in this investigation included ethanol (GC grade, 99.5%; E. Merck, Darmstadt, Germany), which proved to be the only ethanol tested suitable for preparing the internal and calibration standards.

Polypropylene glycol 2025 (30 μl) (E. Merck) was used as antifoaming agent. Sulfur calibration standards included IPS (98%) (Aldrich Chemical Co., Milwaukee, WI), methanethiol (MeSH) (Chem Serve, West Chester, NY), ethanethiol (Chem Serve, West Chester, NY), dimethyl disulfide (DMDS) (>98%, Fluka Chemie, Buchs, Switzerland), dimethyl trisulfide (DMTS) (98%, Haarmann and Reimer, Holzminden, Germany), diethyl sulfide (DES) (97%, E. Merck, Germany), and ethylene sulfide (>98%, Fluka Chemie, Switzerland).

Preparation of Reagents

Internal standard. A 10-ml volumetric flask was half filled with cold ethanol (99.5%, GC grade) and closed with foil. The IPS internal standard (100 μl) was syringed directly into the cold ethanol using a 100-μl precooled syringe, and the flask was filled to the index mark with ethanol (99.5%, GC grade at room temperature). The flask was stoppered and the contents were mixed thoroughly. The IS stock solution was kept at −5°C. Cold ethanol (5 ml, GC grade) was pipetted into a 50-ml volumetric flask and the IS stock solution (100 μl, 5°C) was syringed directly into the alcohol by means of a precooled syringe. The volumetric flask then was filled to the index mark with deionized water (room temperature), stoppered, and thoroughly mixed. This was the IS solution that was added to the samples and calibration standards before analysis. The IS solution was stored in a refrigerator (5°C) and was made up fresh every two weeks.

Calibration Standard Solution

A 5,000-ml flask was filled with deionized water (<10 MΩ/cm), stoppered, and left in the cold room (5°C) to cool overnight. Ethanol (15 ml, GC grade) was added to each of five clean 340-ml beer bottles. The bottles were previously cleaned by thoroughly rinsing three times with deionized water.

The bottles were closed lightly with crowns and put in the cold room to cool. Bottles of deionized water were carbonated in a household "cooldrink" maker (Sodastream, Peterborough, England) to the same level of carbonation (the pressure release valve on the "cooldrink" maker was used to achieve this).

TABLE II
Reproducibility of the Purge-and-Trap/Gas Chromatograph/Sulfur Chemiluminescence Detector System over Eight Runs of the Same Beer[a]

Run No.	Methanethiol	Diethyl Disulfide	Dimethyl Trisulfide
1	1.42	2.14	0.23
2	1.44	2.08	0.31
3	1.62	2.06	0.26
4	1.65	2.35	0.34
5	1.61	1.95	0.30
6	1.21	1.89	0.25
7	1.22	2.06	0.25
8	1.51	2.29	0.24
Mean	1.46	2.10	0.27
SD	0.17	0.16	0.04
Percent relative SD	11.8	7.4	14.4

[a] Results reported in micrograms per liter.

The cold beer bottles were filled to the same level with carbonated deionized water. This level was determined beforehand by pouring 340 ml of deionized water in one of the bottles. The bottles were crowned immediately after filling and stored refrigerated until required.

Calibration Standards

Four 10-ml volumetric flasks were half filled with cold ethanol (GC grade) and closed with foil. The following volumes of compounds were injected directly into the ethanol in separate flasks: DES (150 μl, 0.8362 g/ml), DMS (100 μl, 1.601 g/ml), and ethanethiol (50 μl, 0.8391 g/ml). The mass of each compound was calculated using the given densities in brackets. The purities quoted by the suppliers were used in the calculations.

The flasks were filled to the index marks with ethanol (GC grade) at room temperature, stoppered, and mixed thoroughly. These were the calibration standard stock solutions and were kept at −5°C. Ethanol (5 ml, GC grade) was pipetted into a 50-ml volumetric flask and then half filled with cold deionized water. Using precooled graduated pipettes, each of the calibration stock solutions (100 μl) was pipetted directly into the cold water-ethanol solution in the 50-ml flask. The flask then was filled to the index mark with deionized water at room temperature, stoppered, and mixed thoroughly. This was the calibration standard solution to be added to the calibration standard samples and was kept refrigerated (5°C).

Methanethiol

Because of its low boiling point (6°C), special care had to be taken with the preparation of the MeSH stock solution to obtain accurate calibrations. The MeSH ampoules (1.0 g) were stored at −5°C. Ethanol (25 ml, GC grade) was pipetted into a 250-ml volumetric flask and placed in a crushed ice bath (0–1°C). An ampoule of MeSH (at −5°C) was placed in the volumetric flask (in ice) and broken. The flask then was filled to the index mark with ice cold (1°C) deionized water, stoppered, and mixed thoroughly. This MeSH stock solution was kept tightly stoppered under ice. For the calibration standard solution, a 250-ml volumetric flask was prepared in a similar manner. MeSH stock solution (1.0 ml) was pipetted directly into the ethanol-deionized water mixture using a pipette cooled to −5°C. Cold deionized water (1°C) was used to fill the flask to the index mark, and the flask then was kept under ice in the refrigerator.

A syringe at −5°C was used to inject the calibration standard solution into the calibration samples or into beer for the recovery study. Because an ampoule contained exactly 1.0 g of MeSH, calculations were conducted using this value.

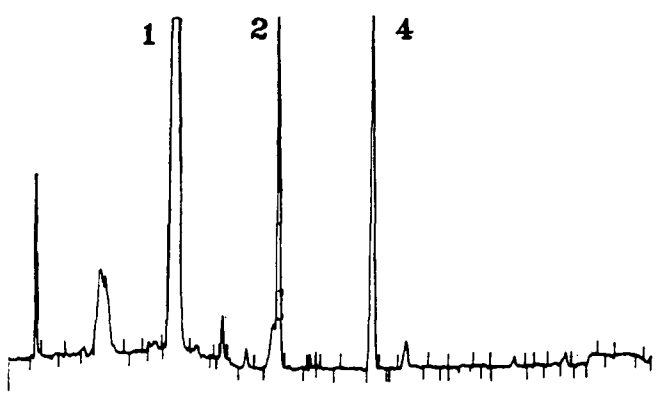

Fig. 7. Sulfur chemiluminescence detector chromatogram of calibration standards made up in distilled water. 1 = Dimethyl sulfide, 2 = diethyl sulfide, 4 = isopropyl sulfide (internal standard).

Addition of Internal Standard and Calibration Standards

The cold sample (beer or calibration standard solution) was clamped firmly in the in-bottle purging device. One of the piercing unions was turned down until the crown was pierced. This was confirmed by pushing a sealed syringe needle through the septum into the bottle headspace. A leak check was performed by placing the device and bottle in cold water and checking for escaping bubbles. The calculated amount of IS (50 μl in 340 ml, 55 μl in 375 ml, and 110 μl in 750 ml) then was injected through the septum directly into the sample by holding the device horizontally. The IS then was mixed thoroughly.

Analytical Procedure

Equilibrated samples were analyzed by purge-and-trap with an HP5880 GC equipped with either a DB-5 or OV-1 megabore column and an FID-SCD detector combination. Data capture and reduction was carried out with an HP3390 integrator and Dapa data system (Dapa Scientific, Kalamunda, Western Australia).

RESULTS AND DISCUSSION

Quantification of the Sulfur Compounds

Authentic sulfur compounds for calibration of the SCD were obtained, and retention time matching in beer was carried out to confirm peak identity. IPS was selected as the IS as it proved to be stable and absent from the beers analyzed. The IS injected directly into the beer or calibration sample was added at a concentration of 2.32 μg/L. Figure 5 shows a typical chromatogram obtained for the calibration sample. The DMS in the calibration mixture was not added but came from the CO_2 (obtained locally from fermentations) used to carbonate the water.

SCD response factors for the volatile sulfur compounds that most likely had an impact on sulfury notes in beer were obtained by performing multipoint calibrations (five different levels of concentration) through addition of the compounds to the calibration medium. The calibration curve for DES is shown in Figure

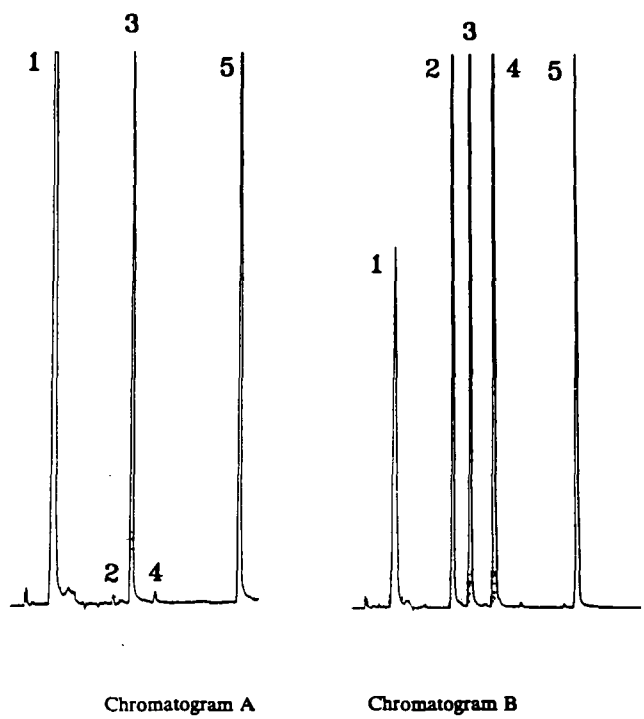

Fig. 8. Comparison of calibration standards made up in **(A)** 99.8% ethanol and **(B)** 99.5% ethanol. 1 = Dimethyl sulfide, 2 = diethyl sulfide, 3 = dimethyl disulfide, 4 = isopropyl sulfide (internal standard), 5 = dimethyl trisulfide.

6. The curves for the other sulfur compounds were similar, and it could be deduced from the graphs that the SCD response for these sulfur compounds was indeed linear, as claimed by the manufacturers (15). This simplified routine calibration of the SCD considerably. An analysis procedure was therefore implemented whereby the daily analysis commenced with analysis of a calibration sample, followed by recalibration whenever it was necessary to adjust response factors. Beer samples could then be analyzed with good day-to-day reproducibility and accuracy.

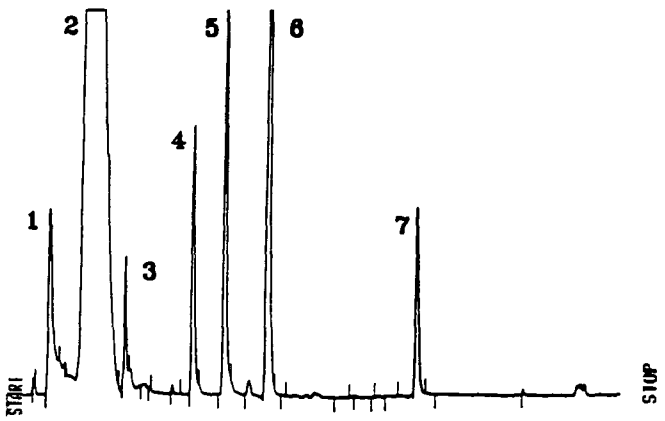

Fig. 9. Sulfur chemiluminescence detector chromatogram of beer with sulfury character. 1 = Methanethiol, 2 = dimethyl sulfide, 3 = ethylene sulfide, 4 = diethyl sulfide, 5 = dimethyl disulfide, 6 = isopropyl sulfide (internal standard), 7 = dimethyl trisulfide.

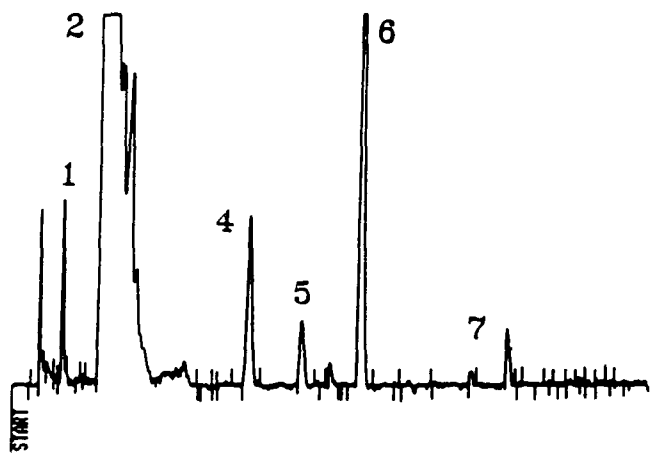

Fig. 10. Sulfur chemiluminescence detector chromatogram of nonsulfury beer. 1 = Methanethiol, 2 = dimethyl sulfide, 3 = ethylene sulfide, 4 = diethyl sulfide, 5 = dimethyl disulfide, 6 = isopropyl sulfide (internal standard), 7 = dimethyl trisulfide.

Recovery Studies for Volatile Sulfur Compounds

Recovery studies for MeSH, DES, DMDS, and DMTS were conducted by adding the calibration mixture (containing the mentioned compounds) to brand A reference beer. The recovery for MeSH was performed separately to prevent sample loss through evaporation. Recoveries for the compounds were as follows: DES, 77.2%; DMS, 89.9%; DMTS, 100.9%; and MeSH, 97.3%.

Reproducibility of the SCD with Calibration Standards

To verify the reproducibility of the purge-and-trap GC-SCD system, 10 consecutive analyses of separately prepared calibration samples (same concentration) were carried out. The mean, standard deviation, and percent relative standard deviation for DES, DMDS, and DMTS are given in Table I. All results are in micrograms per liter.

Reproducibility of the SCD with Beer Samples

To detect small differences in beer sulfur profiles, it is essential that the method should also demonstrate good reproducibility when beer samples are analyzed. It is also imperative that beer from the same batch is used for analysis when the reproducibility of the method is tested.

The beer used by the analytical laboratory as reference samples was used for this study as it was specifically selected to comply with the abovementioned prerequisite. Only MeSH, DES, and DMDS could be detected in all the samples of this beer (brand A) and the respective mean, standard deviation, and percent relative standard deviation were as follows for the compounds: 1.46, 0.17, and 11.8% for MeSH; 2.1, 0.16, and 7.4% for DES; and 0.27, 0.04, and 14.4% for DMDS. All of the results obtained in micrograms per liter are listed in Table II.

Problems Experienced with the SCD

Optimization of the gas flow rates. The operation mode of the SCD requires the FID on which it is mounted to be supplied with a hydrogen-rich flame (5). The manufacturer's recommended flow rates for the HP5880 GC used during this investigation were air at 170–225 ml/min and hydrogen at 325–450 ml/min. This gave an air-to-hydrogen ratio of 1:2 and a maximum total flow rate, including column flow and makeup flow, of 700 ml/min maximum.

Detector response was low at these flow rates and a very noisy baseline was obtained, especially during cryogenic cooling of the oven. A recent paper (15), also using an HP5880 GC, gave a suggestion for better operation by optimizing gas flow rates. Development along these lines gave greatly improved detector response.

The air was eventually supplied at 350 ml/min and the hydrogen

TABLE III
Summary of Sulfur Quality Assurance Results[a] of Three Brands

	Brand								
	A			B			E		
Sulfur	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD
Hydrogen sulfide	ND–1.9[b]	0.4	0.6	ND–1.1	0.3	0.4	0.1–0.9	0.3	0.3
Methanethiol	ND–0.7	0.4	0.2	ND–0.5	0.3	0.1	0.3–0.9	0.5	0.2
Ethylene sulfide	...	ND	...	ND–1.7	0.3	0.4	ND–0.7	0.2	0.2
Diethyl sulfide	0.4–1.0	0.8	0.3	0.5–1.6	0.9	0.4	0.2–1.7	1.0	0.5
Dimethyl disulfide	ND–0.2	0.1	0.1	ND–0.3	0.1	0.1	ND–0.6	0.1	0.2
$\bar{x}$ Sulfury score		1.7			1.5			2.1	

[a] All results reported as micrograms per liter (ppb) except hydrogen sulfide and ethylene sulfide, reported as the ratio of peak area to internal standard peak area.
[b] ND = not detected.

at 360 ml/min for a total flow rate of 750 ml/min. The noisy baseline disappeared. The only logical conclusion was that the previous total flow rate was too low, allowing the SCD's vacuum system to draw in gases from the environment, including the liquid CO_2 used as cryogenic coolant. Because most commercially available CO_2 is obtained from fermentation processes, it contains considerable concentrations of DMS. This observation was confirmed by using CO_2 obtained from one of the local breweries in which the DMS could actually be detected by smell. It was thus established that the total FID flow rate should exceed the maximum pumping rate (in milliliters per minute) of the SCD vacuum system by a substantial amount.

False "flame-on." A sudden loss in detector sensitivity resulting in very small sulfur peaks on the chromatogram was traced to a phenomenon called "false flame-on." It occurred when the FID flame went out but the hydrogen-rich gas in the FID caused the ignitor filament to glow, which in turn helped to partially ionize the eluting compounds. Consequently, only small peaks were seen on the SCD chromatogram. The glowing filament was observed by looking down the FID exhaust chimney. The phenomenon can be easily recognized by observing the SCD digital millivolt readout. This value should be similar, from day to day, when the FID flame is on.

SCD sampling probe. The FID exhaust gas is transferred to the SCD by means of a ceramic probe positioned above the flame tip and attached to the SCD reaction cell and vacuum system through a black perfluoroalkoxy resin line. The manufacturers (Sievers Research, Boulder, CO) recommend that the probe be positioned between 0 and 11 mm above the flame jet tip. Experimentally, it was shown that moving the probe over a range of 4–8 mm from the flame tip did not affect the SCD sensitivity significantly. However, alignment of the probe with the flame jet is important to obtain maximum sensitivity.

FID-SCD transfer line. The black perfluoroalkoxy transfer line is 1.5 m long and, as the name indicates, transfers the FID exhaust gas to the SCD. At the onset of commissioning of the SCD, small negative "dips" were observed on the SCD chromatogram. Because the long transfer line was exposed to the laboratory ambient temperature (24°C), the water-saturated FID exhaust gas condensed. This was confirmed by heating the transfer line to 80°C by means of a tape heater. No negative "dips" could be seen once the line was insulated and kept at 80°C.

Preparation of Calibration Standards

Analyzing and quantifying compounds at extremely low concentrations requires accurate and reproducible sample preparation and calibration techniques. The procedures used during this investigation were based on the method developed for analyzing the esters and alcohols in beer (3). Working at such low concentrations (below the micrograms per liter level) with labile sulfur compounds posed certain unforeseen problems which will be discussed briefly.

Water used for calibration standard dilution. Deionized water (resistance <10 MΩ/cm) was initially selected for the preparation of calibration standards, and good detector sensitivities were obtained even at the lowest calibration concentrations. Due to a shortage of deionized water during the investigation, distilled water was used to prepare calibration standards. When analyzing these standards, it appeared as if some of the compounds were left out of the calibration mixture (Fig. 7). The "missing" compounds however, reappeared on the chromatogram (see Fig. 5) when the same calibration mixture was made up with deionized water. The reaction of the sulfur compounds with microconcentration of heavy metals cannot be excluded as the cause and requires further investigation.

Ethanol used for calibration standard preparation. Gas chromatographic grade ethanol (99.5% purity) was selected to be used for calibration standard preparation as it proved to be the best for the ester and higher alcohol analysis (lowest in higher alcohols). Due to a shortage of GC grade ethanol, analytical grade ethanol (99.7–100%) was used for calibration standard preparation. Certain compounds in the calibration mixture were drastically reduced as can be seen in Figure 8. On analytical comparison, the only obvious difference in the two ethanols was a maximum 0.000002% difference in copper concentration (manufacturer's specifications). This confirmed the sensitivity of the sulfur compounds to heavy metals.

Survey of the Volatile Sulfur Compounds of Different Brands of Beer

Various attributes may contribute toward the character or identity of a specific beer. One of the brands of beer brewed by the SAB, brand E, is known for its sulfury character and preferred by many beer consumers because of this characteristic. The sulfury character of this beer can be distinguished from a nonsulfury beer (brand A) as can be seen in Figures 9 and 10, respectively.

Correlation of Quality Assurance Taste Results with Volatile Sulfur Compound Analytical Data

During the monthly quality assurance taste sessions, beer is judged according to various attributes, one of which is "sulfury." The sulfuriness is ranked on a scale from 0 to 5 (0 = not present and 5 highly objectionable). An average rating of 2 would indicate

TABLE IV
Brand A: Relationship Between Mean Panel Sulfury Scores and Analytical Values

	Brewery				
Data	**I**	**C**	**J**	**N**	**H**
Taste data					
Mean sulfury score	1.5	2.4	1.5	1.7	1.9
Sulfury comments	Dirty	Dirty	...	...	Dirty
Ester	Estery	Estery	Estery	Estery	Estery
Analyses[a]					
H_2S ratio	0.3	1.9	1.2	0.1	TR[b]
MeSH, μg/L	0.5	0.7	0.5	0.3	0.5
ET = S ratio	ND[c]	ND	ND	ND	ND
DES, μg/L	0.9	0.5	0.7	0.9	1.2
DMDS, μg/L	0.2	0.1	0.1	0.1	0.1
DMTS, μg/L	ND	ND	ND	0.5	ND
DMS, μg/L	44	42	40	61	42

[a] H_2S = hydrogen sulfide, MeSH = methanethiol, ET = S = ethylene-sulfide, DES = diethyl sulfide, DMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMS = dimethyl sulfide.
[b] Trace.
[c] Not detected.

TABLE V
Brand E: Relationship Between Mean Panel Sulfury Scores and Analytical Values

	Brewery			
Data	**E**	**N**	**M**	**H**
Taste data				
Sulfury score	2.1	1.8	1.8	1.8
Sulfury remarks	Dirty	...	Dirty	...
Ester	Estery	Estery	Estery	Estery
Analyses[a]				
H_2S ratio	0.1	0.1	0.3	0.2
MeSH, μg/L	0.5	0.5	0.5	0.7
ET = S ratio	0.1	0.7	0.4	0.1
DES, μg/L	1.4	0.8	0.8	1.5
DMDS, μg/L	0.1	ND[b]	0.1	ND
DMTS, μg/L	0.5	ND	ND	ND
DMS, μg/L	35	46	46	45

[a] H_2S = hydrogen sulfide, MeSH = methanethiol, ET = S = ethylene sulfide, DES = diethyl sulfide, DMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMS = dimethyl sulfide.
[b] Not detected.

a noticeable sulfury note. The descriptors used by the profile taste panel to describe sulfuriness were used as such in the correlations.

The primary objective of this investigation was to develop a method with which analytical data could be obtained that could be correlated with monthly quality assurance profile taste results. Once this method demonstrated that the volatile sulfur compounds could be quantified, the analytical data was reviewed in the light of the taster assessments.

As this investigation was secondary to the normal quality assurance taste and analysis programs, only when spare samples were available was this study conducted. The sample allocation was further restricted, as this study was confined to bottled beer and on many occasions canned beer was used for the taste assessment.

A summary of the concentration ranges of volatile sulfur compounds found in the beers subjected to quality assurance testing can be found in Table III. Because H_2S has such a strong flavor impact (18), and because of the difficulty in quantification, the concentration was expressed as the ratio of the peak area to that of the internal standard. Similarly, because of the uniqueness of ethylene sulfide, the concentration has been expressed in the same manner as the H_2S. The term "relative" concentration will be used when discussing their values.

It is well established that estery notes tend to mask other off-flavors. For this reason, the tasters' ester comments are included in the tables. The effect of beer esteriness on undesirable sulfur flavors has not been studied, but as it can lessen the effect of undesirable hop flavors (18), the possible impact on sulfury notes should not be discounted.

The quality assurance taste data for sulfury notes as well as the analytical data obtained for these beers are listed in Tables IV–VIII. It appeared that the tasters only reacted to sulfury notes when the average sulfury score exceeded 1.8. When conducting a study of this nature, one has to be cognizant of the fact that objective analytical results are compared with subjective taste data, and certain discrepancies will occur.

The beer from brewery C (Table IV) was considered very sulfury, which most likely was caused by the combined effect of both high H_2S and MeSH concentrations. A strong sulfury note (1.9) also was found in beer from brewery H, which was possibly caused by the high concentration of DES (exceeded the flavor threshold of 1 μg/L). Despite the high H_2S concentration in the beer from brewery J, there was no taster reaction, possibly because of the masking effect of the concomitant estery note. The high DMS concentration in the beer from brewery N apparently had little impact on undesirable sulfuriness.

The higher perceived sulfuriness of brand E (Table V) is reflected in the higher mean sulfury score accorded by the panel. However, analytically, the concentrations of the volatile sulfur compounds did not support this opinion. The high score accorded to the beer from brewery E could possibly be attributed to the amount of DMTS present in this beer.

The dirty sulfury note found on the beer from brewery A (Table VI) could not be explained by the analytical data available.

Both the H_2S and DES concentrations on the beer from brewery I were high (Table VII). However, the true sulfury character of this beer could have been overridden by the strong "yeasty" flavor present.

The beer from brewery E (Table VIII) stood out as being excessively sulfury, and the concentrations of MeSH and DES were high. In addition, the concentration of H_2S was high. However, the analytical data did not fully account for the generally high sulfur ratings accorded to this brand. This was even more evident in the case of beer from brewery H.

TABLE VI
Brand B: Relationship Between Mean Panel Sulfury Scores and Analytical Values

	Brewery				
Data	**G**	**I**	**J**	**A**	**C**
Taste data					
Mean sulfury score	1.4	1.4	0.8	1.5	1.3
Sulfury comments	...	...	...	Dirty	...
Analyses[a]					
H_2S ratio	ND[b]	ND	0.1	0.1	0.2
MeSH, μg/L	0.3	0.3	0.3	ND	0.3
ET = S ratio	ND	0.1	0.4	0.1	ND
DES, μg/L	1.3	0.9	0.8	0.9	0.5
DMDS, μg/L	ND	0.3	ND	ND	ND
DMTS, μg/L	ND	0.6	ND	ND	ND
DMS, μg/L	36	43	34	46	42

[a] H_2S = hydrogen sulfide, MeSH = methanethiol, ET = S = ethylene sulfide, DES = diethyl sulfide, DMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMS = dimethyl sulfide.
[b] Not detected.

CONCLUSIONS

The analytical procedure developed for the analysis of volatile flavor-active sulfur compounds in beer is very sensitive and reproducible provided that certain precautions are strictly noted. First, the flow rates of the flame gases of the FID should be

TABLE VII
Brand A: Relationship Between Mean Panel Sulfury Scores and Analytical Values

	Brewery			
Data	**G**	**J**	**H**	**I**
Taste data				
Sulfury score	1.1	1.7	1.7	1.9
Sulfury remarks	...	...	...	"Yeasty"
Ester	...	...	Estery	...
Analyses[a]				
H_2S ratio	ND[b]	0.1	0.2	0.3
MeSH, μg/L	0.2	ND	0.5	0.4
ET = S ratio	ND	ND	ND	ND
DES, μg/L	0.7	0.4	0.6	0.9
DMDS, μg/L	0.2	ND	0.2	ND
DMTS, μg/L	ND	ND	ND	ND
DMS, μg/L	38	32	43	43

[a] H_2S = hydrogen sulfide, MeSH = methanethiol, ET = S = ethylene sulfide, DES = diethyl sulfide, DMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMS = dimethyl sulfide.
[b] Not detected.

TABLE VIII
Brand E: Relationship Between Mean Sulfury Scores and Analytical Values

	Brewery			
Data	**J**	**H**	**M**	**E**
Taste data				
Sulfury score	2.2	2.5	1.7	2.6
Sulfury remarks	...	Dirty, unpleasant	...	"Yeasty"
Analyses[a]				
H_2S ratio	0.3	0.4	0.2	0.6
MeSH, μg/L	0.4	0.4	0.3	0.7
ET = S ratio	ND[b]	0.1	ND	0.1
DES, μg/L	0.6	1.2	1.0	1.2
DMDS, μg/L	0.2	ND	0.1	0.3
DMTS, μg/L	ND	ND	ND	ND
DMS, μg/L	40	40	34	33

[a] H_2S = hydrogen sulfide, MeSH = methanethiol, ET = S = ethylene sulfide, DES = diethyl sulfide, DMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMS = dimethyl sulfide.
[b] Not detected.

adjusted carefully to ensure optimum sensitivity of the SCD.

The highest quality ethanol (GC grade) and deionized water should be used for the preparation of the calibration standard. It is also critical to keep the SCD vacuum system functioning while the FID flame is on to prevent fouling of the SCD sampling probe.

Although it proved to be difficult to find good correlation between subjective taste data and objective analytical data, certain correlations were found. The roles played by H_2S, MeSH, DES, and to a lesser extent, DMTS, were obvious in some cases of extreme sulfuriness in beer. There is a relationship between the DES and H_2S concentrations in beer flavor. One of the most significant findings is that one brand considered sulfury by the panel did not contain any of the measured sulfur compounds in excessive concentrations. This emphasizes that either an important sulfur compound has not been detected in this investigation or that other substances mask sulfur character to a greater extent in some brands than in others. A synergistic effect was evident at times. Masking of sulfury notes by other flavors, such as esteriness, also had an undetermined effect, which should be further investigated.

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