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Low-Level Sulfur Compounds in Beer by Purge and Trap with a Pulsed Flame Photometric Detector (PFPD)

Application Note 16181100

Keywords
Beer
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Sulfur

Introduction

The presence or absence of volatile sulfur compounds in beer can have a significant impact on the quality of the final brewing product. Trace levels of specific sulfur-containing compounds often are responsible for imparting the characteristic pleasant taste and aroma of the beer, while excess concentrations or absence of some of those same compounds can be responsible for an undesirable odor or taste. It is essential for the brewing industry worldwide to be able to accurately identify and quantify the specific sulfur compounds present to detect deviations in the brewing process.

Analysis of the sulfur compounds in beer is typically done by GC methodology. The most common sample introduction techniques are static headspace, dynamic headspace (also know as purge and trap), and solid phase micro extraction (SPME). The flame photometric detector (FPD) and the sulfur chemiluminescence detector (SCD) are frequently cited for this type of analysis, but both have significant drawbacks. The atomic emission detector (AED) and mass spectrometer (MS) are usually not chosen because of their high cost and lack of the necessary sensitivity, respectively.

The pulsed flame photometric detector (PFPD) is increasingly being accepted as the standard GC detector for sulfur compounds in the European brewing industry and is gaining acceptance in the United States as well. The PFPD does not suffer from the sensitivity, selectivity, and quenching problems of the FPD, and it is significantly more stable and requires less maintenance than the SCD. One advantage that the PFPD has over the other sulfur selective detectors is the additional time domain information that the PFPD provides for the elemental emission profile. The time domain emissions yield increased sensitivity (10X), increased selectivity (up to 10^3 or more), equimolar sulfur response, and reduced gas consumption (~$^1/_{10}$).

The dynamic headspace, or purge-and-trap (P&T) introduction technique, is often preferred because of its ability to strip all of the volatile sulfur compounds out of the aqueous matrix and concentrate them onto a sorbent trap prior to thermal desorption onto the GC column, thus maximizing sensitivity. The Silicosteel® coating on the entire sample pathway reduces the adsorption and decomposition of reactive sulfur compounds, which are sometimes difficult to recover at low concentrations.

This application note demonstrates the superb low-level performance possible in a system that couples both the OI Analytical Model 4560 Sample Concentrator and the Model 5380 Pulsed Flame Photometric Detector for the analysis of sulfur compounds in beer. It also confirms the increased analyte sensitivity that can be achieved using the OI Analytical Model 4551A Autosampler and the Model 4560 Sample Concentrator.

Experimental

The OI Analytical Model 4560 Purge-and-Trap Sample Concentrator was configured with the Model 4551A Autosampler and an Agilent 6890 Gas Chromatograph (GC) to facilitate fully automated sample transfer and analysis. All interior sample transfer lines in the purge and trap are Silicosteel coated to provide an inert sample pathway. The optional Infra-Sparge™ Sample Heater was used to accelerate the purge efficiency of polar compounds, and the patented Cyclone Water Management™ feature removed most of the water transferred from the sample matrix to the trap during sparging with no noticeable detrimental effect on the sulfur compounds. A complete description of the equipment configuration and operating conditions is listed in Tables 1, 2, and 3.

Table 1. Instrument Settings for the OI Analytical Model 4560 Sample Concentrator Optimized for Analysis of Volatile Sulfur Compounds in Beer

OI Analytical Model 4560 Sample Concentrator	
Sample Size	5-mL sample in a 25-mL fritted sparger
Trap	Tenax®
Sample Temp	45°C
Purge Time/Temp	11 min at ambient
Desorb Time/Temp	0.5 min at 180°C
Bake Time/Temp	10 min at 200°C
Clean Water Rinses	2

Table 2. Instrument Settings for the Agilent 6890 GC

Agilent 6890 GC	
Inlet	200°C; split 100:1
Column	J&W GS-GasPro®, 30 m x 0.32 mm I.D.
Oven	80°C for 2.5 min 15°C/min to 260°C Hold for 1 min
Carrier Gas	He, 1.2 mL/min constant flow

Table 3. Configuration for the OI Analytical Model 5380 PFPD Optimized for the Detection of Sulfur

OI Analytical Model 5380 PFPD	
Temperature	250°C
Combustor	2 mm
Filter	BG-12
Photomultiplier	R1924
Gas Flows	H_2: 12.5 mL/min Air 1: 10 mL/min Air 2: 10 mL/min
Pulse Frequency	3.3 Hz
Sulfur Gate	6 to 25 msec
Signal #1	Quadratic Mode
Signal #2	Linear Mode

A stock solution containing nine volatile sulfur compounds of interest to the brewing industry was prepared. The stock solution was used to generate calibration standards, as well as a midrange standard to measure instrument repeatability. The standards were prepared in 5% ethanol to mimic the beer matrix. The beers were cooled to 5°C and transferred to 40-mL VOA vials by overfilling to the point that the vials contained only liquid and no foam. Approximately five drops of Dow Corning Antifoam 1520 (diluted 1:10 with water) were added to each standard and sample vial before sealing the vials. No other sample preparation was necessary. The vials were placed in the autosampler tray and analyzed by an automated GC sequence. A 5-mL aliquot of each standard or sample to be analyzed was transferred to the 25-mL fritted sparger of the sample concentrator and was analyzed as described above. Each analysis was followed by two clean water rinses. Except where noted, all concentrations are reported as parts per billion sulfur (ppbS). Note that even at these low concentrations, the analyses were run with a split ratio of 100:1 to ensure that the PFPD did not become saturated due to an excess amount of sulfur going to the detector.

Results and Discussion

Repeatability

Repeatability of the PFPD response (by area counts) for the nine sulfur compounds over 20 replicate analyses ranged from 3.0% (methyl sulfide) to 6.3% (ethylthioacetate). The overlaid chromatograms from ten of the 20 replicate analyses are shown in Figure 1. Ethanethiol (ETHIOL) is a polar compound, is very soluble in water, and was not detected at the concentration tested. Percent Relative Standard Deviations (%RSD) for all nine sulfur compounds are detailed in Table 4.

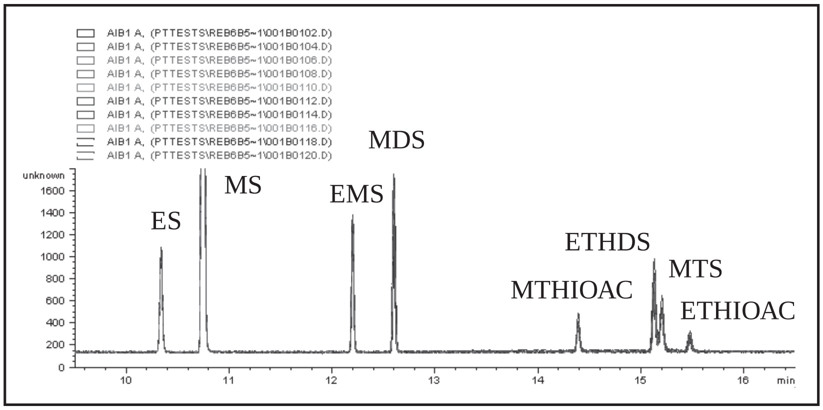


Figure 1. Overlaid Chromatograms From Ten of 20 Replicate Analyses of a Low- to Midrange Sulfur Standard Analyzed Using P&T with PFPD. RSDs ranged from 3.0% to 6.3%.

Table 4. Repeatability of Response Over 20 Replicate Analyses for Nine Light Molecular Weight Sulfur Compounds by Purge and Trap with PFPD

Compound Name	**Abbreviation**	**Concentration (ppbS)**	**%RSD (n = 20)**
Ethanethiol	ETHIOL	0.260	*
Ethylene sulfide	ES	0.375	4.2
Methyl sulfide	MS	0.290	3.0
Ethyl methylsulfide	EMS	0.355	4.4
Methyl disulfide	MDS	0.235	3.1
Methylthioacetate	MTHIOAC	0.355	4.6
Ethyl disulfide	ETHDS	0.225	5.1
Methyl trisulfide	MTS	0.275	5.7
Ethylthioacetate	ETHIOC	0.200	6.3

*Ethanethiol is a polar, water-soluble compound and was not detected at this concentration.

Calibration

The PFPD produces a quadratic response for sulfur over approximately two and a half orders of sample concentration, which represents approximately five orders of magnitude signal response. If desired, automated linearization of the quadratic output signal is possible using the Model 5380 PFPD software. Enabling the square root function when setting up the electronic gates for sulfur activates the calculation of the square root of the output signal. This feature is used to linearize the quadratic response signal associated with sulfur, thus providing a useful linear calibration curve. For this experiment, data were acquired in both the quadratic and linear modes simultaneously for comparison. Chromatograms from the midrange calibration standard are shown in Figure 2.

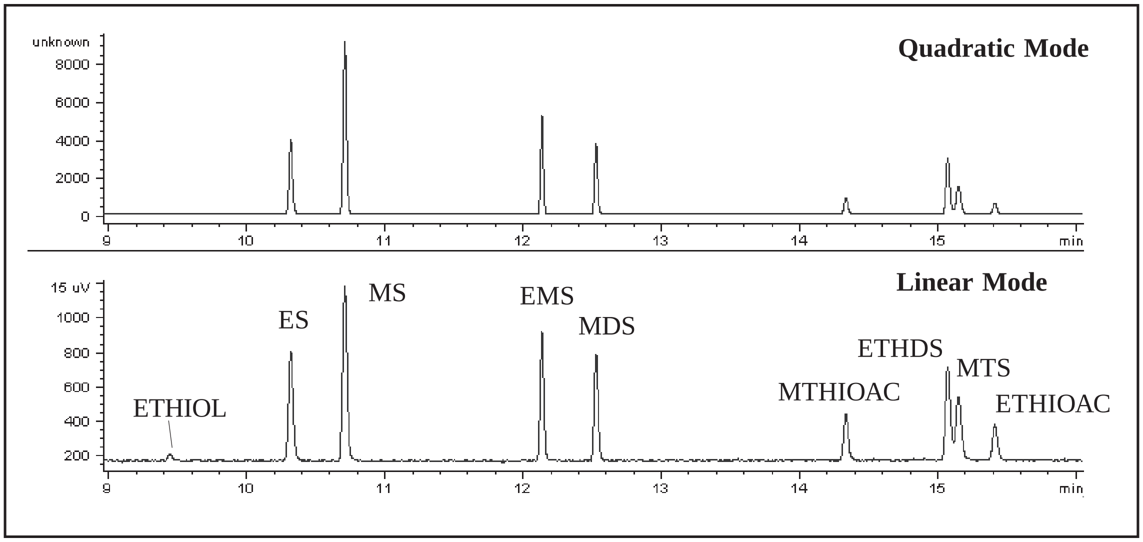


Figure 2. Chromatograms From a 5-mL Aliquot of the Midrange Calibration Standard, Split 100:1. Concentrations of Each Compound Range From 0.4 to 0.75 ppbS. Signal #1 was Acquired in the Quadratic Mode, and Signal #2 was Acquired in the Linear Mode.

The original stock standard was used to prepare calibration solutions at six different concentration levels bracketing the expected range, from approximately 0.1 ppbS to 15 ppbS. Because of the different purge efficiencies for each compound, not all analytes were calibrated over all six concentrations. Calibration statistics for all nine compounds are reported in Table 5. An example of both the quadratic and linear calibration curves for ethyl disulfide are shown in Figure 3.

Table 5. Calibration Statistics for Nine Volatile Sulfur Compounds by Purge and Trap with PFPD

Compound	**Calibration Range (ppbS)**	**R^2 Quadratic Mode**	**R^2 Linear Mode**
ETHIOL	0.52–10.4	0.9996	0.9994
ES	0.15–1.50	0.9996	0.9991
MS	0.12–1.16	1.0000	0.9996
EMS	0.14–1.42	0.9998	0.9990
MDS	0.09–0.94	0.9998	0.9991
MTHIOAC	0.14–7.10	0.9998	0.9997
ETHDS	0.90–0.90	0.9990	0.9982
MTS	0.11–1.10	0.9998	0.9998
ETHIOAC	0.20–4.00	0.9990	0.9998

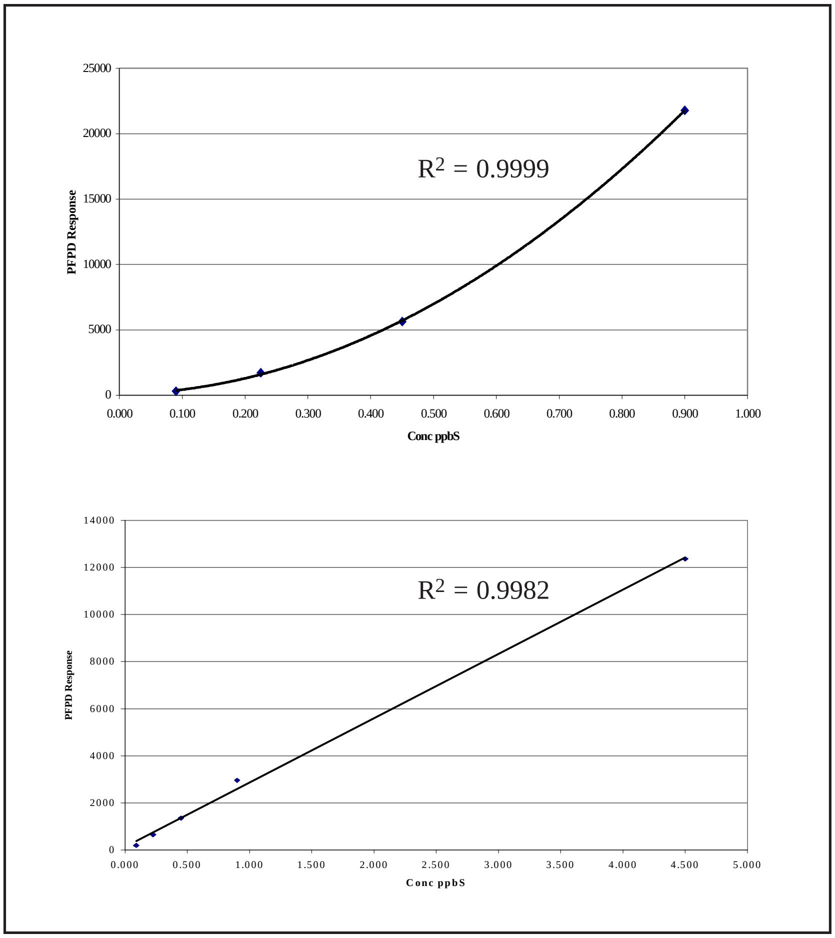
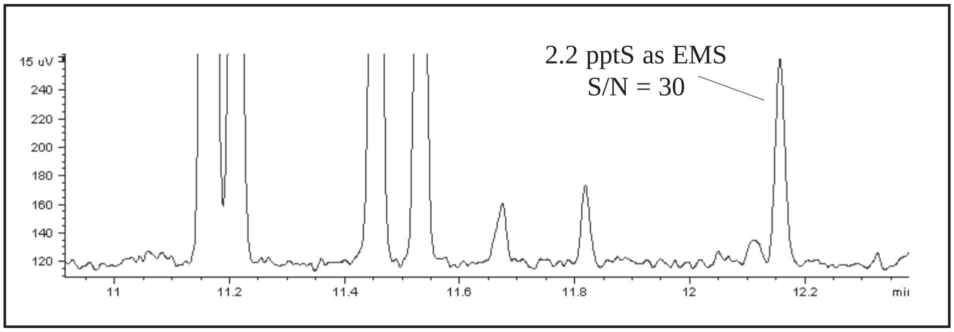


Figure 3. Quadratic and Linear Calibration Curves for Ethyl Disulfide

Ultimate Sensitivity

Sample size, injection split ratio, and calibration range were all selected to bracket the expected concentration of analytes in the actual beer samples. However, because of the small sample size (5 mL) and the high split ratio (100:1), there was sufficient capacity left to optimize for ultimate sensitivity. To demonstrate detection of a low-level compound, a sample containing 0.022 ppbS (22 pptS) as EMS was diluted 1:10 with clean water, giving an EMS concentration in the single-digit ppt range (2.2 pptS). A 25-mL aliquot was analyzed with a 10:1 injection split ratio, and the resulting EMS peak had a signal-to-noise ratio of 30. (See Figure 4.)



2.2 pptS as EMS
S/N = 30

Figure 4. Chromatogram of a Sample Containing 2.2 pptS as EMS. The Analysis was Done Using a 25-mL Aliquot and a Split Ratio of 10:1.

Beer Samples

Eight different varieties of beer were analyzed under the conditions described, including five products from commercial breweries, one test sample, and two examples of home brewed beer. All eight chromatograms had a similar distribution of peaks corresponding to many of the calibration compounds as determined by retention time (RT), as well as one or more unknowns. Chromatograms for all eight beers are shown in Figure 5. Calculated concentrations of selected sulfur compounds are shown in Table 6.

Table 6. Calculated Concentrations of Selected Sulfur Compounds in the Five Commercial Beers, One Test Sample, and Two Home Brew Beers Tested. Concentrations are Reported as Parts Per Billion of the Individual Compound.

Beer	**ES Conc. (ppb)**	**MS Conc. (ppb)**	**MDS Conc. (ppb)**	**MTHIOAC Conc. (ppb)**	**MTS Conc. (ppb)**
Miller	0.45	0.21	0.18	5.54	
Miller Lite	0.65	0.27	1.49	5.79	
Budweiser		0.30	0.84	4.07	
Samuel Adams	0.31	0.39	2.19	1.05	
Test Sample		0.20	0.35	2.94	0.10
Shiner Bock	0.51	0.47	0.53	4.70	
Honey Wheat		0.11	0.62	0.73	
Barringer Stout	0.26	0.13	4.84	9.52	0.75

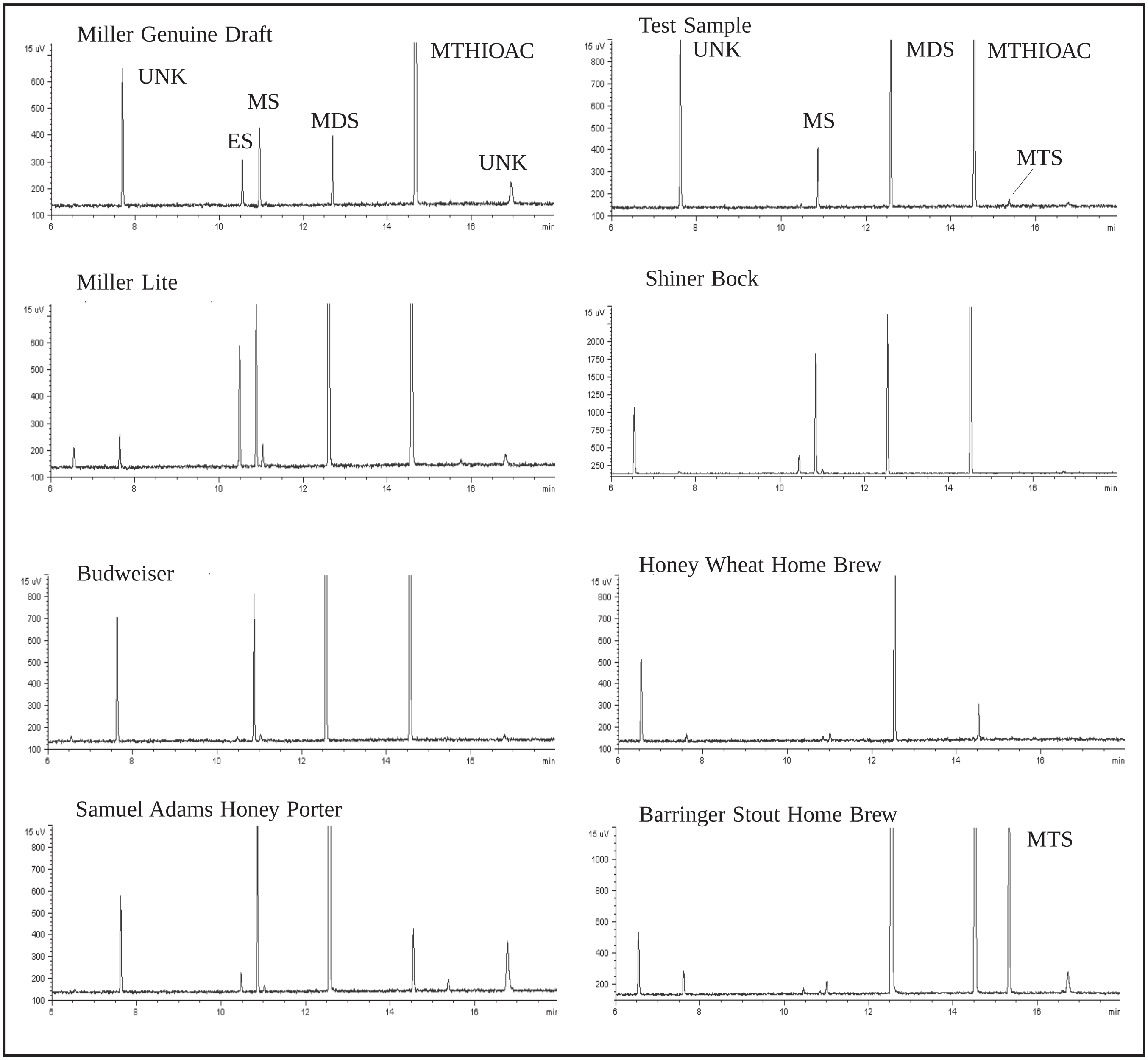


Figure 5. Chromatograms of Five Commercially Brewed Beers, One Test Sample, and Two Home Brewed Beers Showing Similar Peak Patterns. Concentrations of Identified Peaks Range From 0.1 ppb to 9.5 ppb.

Carryover Test

After each sample, the sample transfer pathway from the autosampler to the sample concentrator, the sparge needle, and the fritted sparge vessel were rinsed twice with clean rinse water. Blank water was run at random intervals between samples to check for carryover. A chromatogram of the last blank run demonstrating virtually no carryover from the beer is shown in Figure 6.

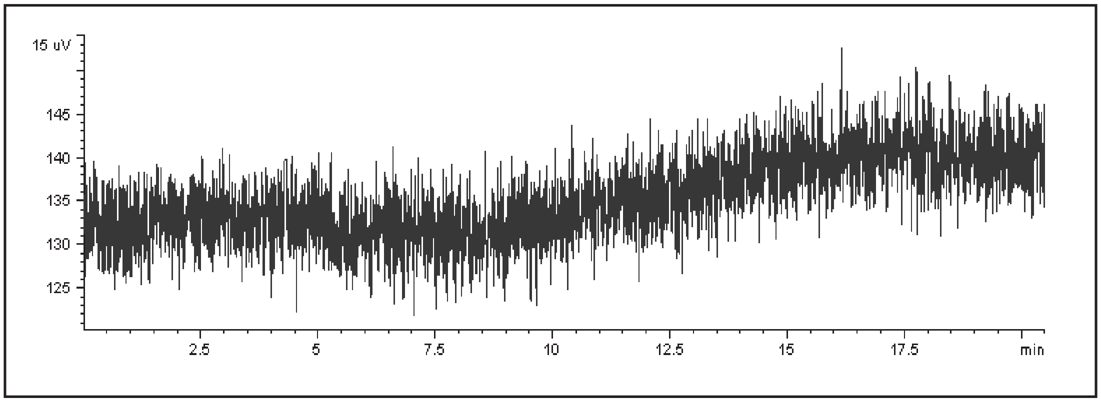


Figure 6. Chromatogram of 5-mL Blank Water Analyzed Immediately Following a High-Level Beer Sample Demonstrating Virtually No Carryover

Conclusion

When coupled with the OI Analytical Model 4560 Sample Concentrator, the PFPD offers superior sensitivity and selectivity for the analysis of low-level sulfur compounds in beer. Overall system performance was excellent even at sub-ppb levels, with repeatability (measured as %RSD) at 6.3% or better for the volatile sulfur analytes tested. Calibration curves can be generated in either the linear or quadratic modes, both with equally outstanding R^2 values. Ultimate sensitivity in the single-digit pptS range can be achieved using this configuration by making only minor modifications to the analytical conditions.

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