O·I·Analytical

Determination of Volatile Sulfur Compounds in Beverages Using Static Headspace and Pulsed Flame Photometric Detection

Application Note 15020800

Keywords
Beer
Beverages
Coffee
Flavor
Fragrance
PFPD
Rum
Static Headspace
Sulfur
Wine

Introduction

The presence or absence of sulfur compounds in beverages can have a significant impact on product quality. Specific sulfur-containing compounds often are responsible for imparting the characteristic pleasant taste and aroma of some beverages (e.g., beer, wine, or rum). Excess concentrations of some of those same compounds or the presence of unexpected sulfur compounds can be responsible for an undesirable odor or taste and can indicate product degradation. In addition, sulfur compounds are sometimes used as a measure of deviations in the brewing and manufacturing processes. Offending sulfur compounds can be native in the starting materials as in some alcoholic beverages, can be introduced with the CO_2 as in carbonated beverages, or can even migrate into the beverage from the packaging materials or storage containers. Even very low concentrations of sulfur compounds can greatly affect the flavors and fragrances, and because their presence is noticeable even at very low, sub-ppm levels, accurate detection and quantitation is an important manufacturing quality control step.

Analysis of the sulfur compounds in beverages is typically done by GC methodology. Some of the common sample introduction techniques include direct injection, extraction followed by direct injection, static headspace, dynamic headspace, solid phase micro extraction (SPME), and the new stir bar sorptive extraction (SBSE) technique (Twister™) recently introduced by Gerstel GmbH & Co. Some of the detectors commonly used for selective sulfur analysis are FPD, PFPD (pulsed flame photometric detector), SCD, AED, MS, and tandem configurations such as PFPD/MS.

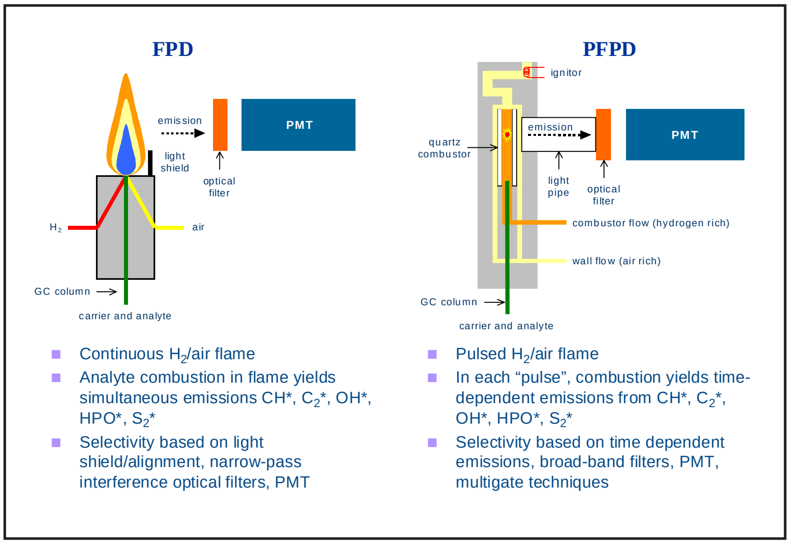
The PFPD has advantages over the other sulfur selective detectors because of the additional time domain information that it provides for the elemental emission profile. The time domain emissions yield increased sensitivity (10X), increased selectivity (up to 10^3 or more), equimolar sulfur response, and reduced gas consumption (~1⁄10). Figure 1 shows the principle of PFPD operation as compared to a conventional FPD. The time separated emission profiles for sulfur and phosphorus (2-mm combustor) are shown in Figure 2.

The static headspace introduction technique is very desirable and effective for beverage analyses because there is little or no sample preparation involved. Low concentrations of the volatile components are introduced to the GC without the nonvolatile materials (e.g., sugars, carbohydrates, preservatives, etc.) contaminating the GC inlet, and the entire process can be automated for unattended operation. Additionally, samples that are prone to foaming (e.g., beer) can be handled easily using the headspace method.

This application note will detail the analysis of a variety of beverages using the static headspace technique coupled with the detection of volatile sulfur components using the PFPD. It will also show how the PFPD used in tandem with a mass spectrometer (MS) provides several advantages not realized by using either detector alone. Some results using the Gerstel Twister technique for sample extraction will also be shown.

Sulfur Detection by Headspace/PFPD

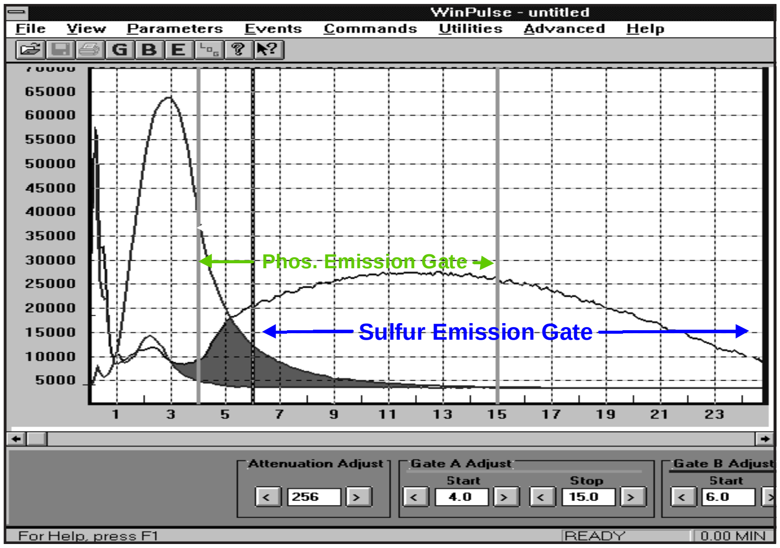
The OI Analytical Model 4632M Headspace Autosampler, with its 32-place sampling tray, was mounted directly on an Agilent 6890 Gas Chromatograph (GC). Gas pressurization lines were plumbed through a programmable Agilient auxiliary pressure control module. The OI Analytical Model 5380 Pulsed Flame Photometric Detector (PFPD) was configured for selective sulfur analysis. Complete operating conditions for the static headspace autosampler, the GC, and the PFPD are detailed in Tables 1 and 2.



- Continuous H_2/air flame
- Analyte combustion in flame yields simultaneous emissions CH*, C_2*, OH*, HPO*, S_2*
- Selectivity based on light shield/alignment, narrow-pass interference optical filters, PMT

- Pulsed H_2/air flame
- In each "pulse", combustion yields time-dependent emissions from CH*, C_2*, OH*, HPO*, S_2*
- Selectivity based on time dependent emissions, broad-band filters, PMT, multigate techniques

Figure 1. Principle of PFPD Operation Compared to Conventional FPD



Attenuation Adjust: 256

Gate A Adjust: Start 4.0, Stop 15.0

Gate B Adjust: Start 6.0

Figure 2. PFPD Emission Waveform Showing the Time Delayed Emission Profiles for Sulfur and Phosphorous

Table 1. Instrument Settings for the OI Analytical Model 4632M Headspace Autosampler Optimized for Analysis of Light Sulfur Compounds in Beverages

Headspace Parameter	Setting
Headspace Autosampler	OI Analytical Model 4632M
Incubation Time	30 minutes
Incubation Temperature	80°C
Sample Valve Temperature	110°C
Vial Agitation	ON
Vial Pressurization	2 seconds
Vial Equilibration	5 seconds
Sampling Time	6 seconds
Vent Purge Delay	6 seconds
Injection Time	30 seconds

Table 2. Instrument Settings for the Agilent 6890 GC and OI Analytical PFPD

GC Parameter	Setting
Gas Chromatograph	Agilent 6890 with EPC
Column	J&W DB-5MS, 30 m, 0.25 mm I.D., 1.0 µm film thickness
Column Mode	Constant flow, 1 mL/minute
Inlet Temperature	250°C
Inlet Mode	Pulsed split (20 psi for 0.5 minutes)
Split Ratio	9:1
Carrier Gas	Helium
Oven Program	35°C for 5 minutes 15°C/minute to 280°C Hold for 2 minutes
Detector Parameter	**Setting**
Detector	OI Analytical Model 5380 PFPD
Temperature	250°C
Analysis Mode	Sulfur
Combustor	2 mm
PMT	R1924
Optical Filter	BG-12
Sulfur Gate	6 to 24.9 mseconds

A solution of nine volatile sulfur compounds was prepared from stock standard in 5% ethanol to mimic the beer and wine matrices. Concentrations of the individual sulfur components in the solution ranged from 5.2 ppbS to 13.4 ppbS. (All concentrations in this application note are reported as ppb of sulfur, rather than ppb of compound.) Ten 5-mL aliquots of the solution were transferred to headspace vials, placed in the autosampler tray, and analyzed by an automated GC sequence. Reproducibility of the PFPD response (by area counts) for the nine sulfur compounds at this low ppb level ranged from 3.9% (dimethyl disulfide) to 5.7% (heptyl mercaptan). The overlaid chromatograms from the ten replicate analyses are shown in Figure 3. Percent Relative Standard Deviation (%RSD) for the nine sulfur compounds over ten replicate runs are detailed in Table 3.

The original stock standard was used to prepare calibration solutions at four different concentration levels, from approximately 1 ppbS to 50 ppbS. The four solutions were analyzed using the conditions described in Tables 1 and 2, and quadratic calibration curves were generated using peak areas. Details of the calibration results are shown in Table 4. The quadratic calibration curve for methyl sulfide is shown in Figure 4.

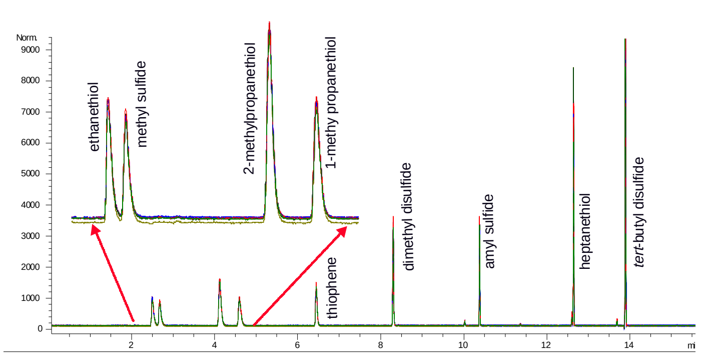


Figure 3. Overlaid Chromatograms From Ten Replicate Analyses of a Nine-Component Sulfur Standard Analyzed Using Static Headspace with PFPD. RSDs Ranged from 3.9% to 5.7%

Following calibration, a variety of beers, wines, and liquors were analyzed using the analytical conditions described earlier. Concentrations were calculated from the quadratic calibration curves using peak areas. The sulfur selective chromatograms for all beverages tested, including calculated analyte concentrations, are shown in Figures 5, 6, 7, and 8.

Table 3. Reproducibility of Response over Ten Replicate Analyses for Nine Light Molecular Weight Sulfur Compounds by Static Headspace Technique with PFPD. Compound Concentrations are Reported as ppbS

	Ethanethiol	**Methyl Sulfide**	**2-Methyl Propanethiol**	**1-Methyl Propanethiol**	**Thiophene**	**Dimethyl Disulfide**	**Amyl Sulfide**	**Heptyl Mercaptan**	***tert*-Butyl Disulfide**
Conc. (ppbS)	10.8	10.4	7.2	9.3	9.4	13.4	5.9	5.2	7.3
Run 1	2903.4	2580.4	5190.9	3440.4	3101.1	4709.2	3295.2	6648.7	45619.0
Run 2	3103.7	2625.6	5473.7	3584.7	3181.8	4842.6	3450.2	7287.4	48688.3
Run 3	3064.3	2599.3	5557.3	3705.2	3223.9	4923.5	3481.5	7413.3	47985.4
Run 4	2966.5	2607.7	5356.1	3532.3	3148.4	4771.9	3422.2	7506.8	46453.0
Run 5	2812.0	2510.1	4903.6	3268.6	2968.9	4631.5	3155.6	7366.4	43249.0
Run 6	2804.7	2427.5	4982.3	3239.2	2967.0	4550.9	3201.3	6956.9	43526.1
Run 7	3003.3	2604.6	5371.8	3550.5	3133.4	4881.9	3435.4	7525.2	46125.6
Run 8	2802.5	2436.2	4950.3	3273.3	2981.8	4701.0	3234.1	6469.4	43570.5
Run 9	3064.9	2764.5	5399.9	3642.6	3312.2	5159.4	3561.9	6631.4	45949.4
Run 10	3049.7	2685.6	5645.5	3533.3	3273.9	5044.7	3648.5	6857.2	49387.6
Avg	*2957.5*	*2584.2*	*5283.1*	*3477.0*	*3129.2*	*4821.7*	*3388.6*	*7066.3*	*46055.4*
StDev	*118.5*	*104.2*	*263.2*	*165.2*	*125.2*	*187.8*	*161.7*	*399.9*	*2174.9*
%RSD	*4.0*	*4.0*	*5.0*	*4.8*	*4.0*	*3.9*	*4.8*	*5.7*	*4.7*

Table 4. Calibration Range (as ppb sulfur) and Quadratic R^2 Values for Nine Volatile Sulfur Compounds by Static Headspace with PFPD

Compound Name	**Calibration Range (ppbS)**	**Quadratic R^2 Value**
Ethanethiol	1.08–54.0	0.9987
Methyl sulfide	1.04–52.0	0.9999
2-Methyl propanethiol	0.72–36.0	0.9995
1-Methyl propanethiol	0.93–46.5	0.9993
Thiophene	0.94–47.0	0.9994
Dimethyl disulfide	1.34–67.0	0.9999
Amyl sulfide	0.59–29.5	0.9994
Heptyl mercaptan	0.52–26.0	0.9981
tert-Butyl disulfide	0.73–36.5	0.9996

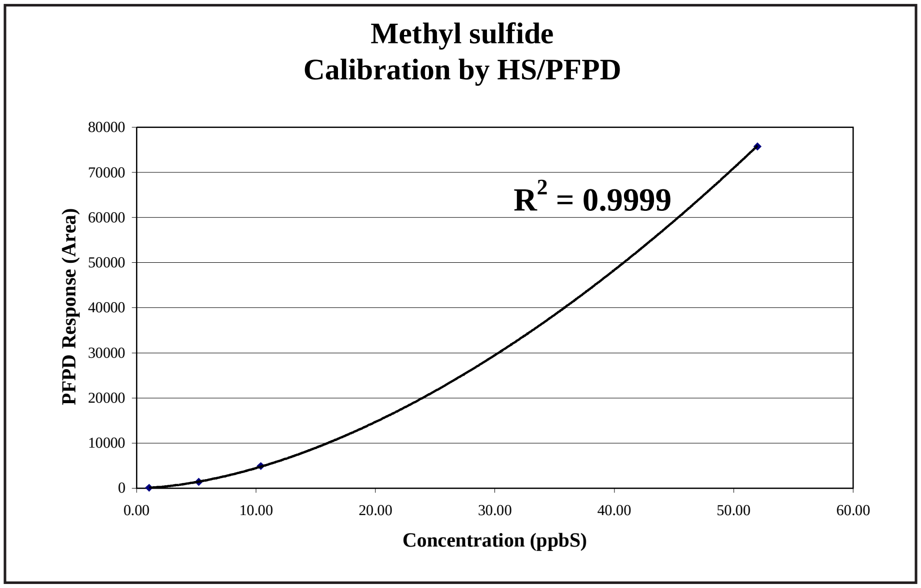


Figure 4. Quadratic Calibration Curve for Methyl Sulfide

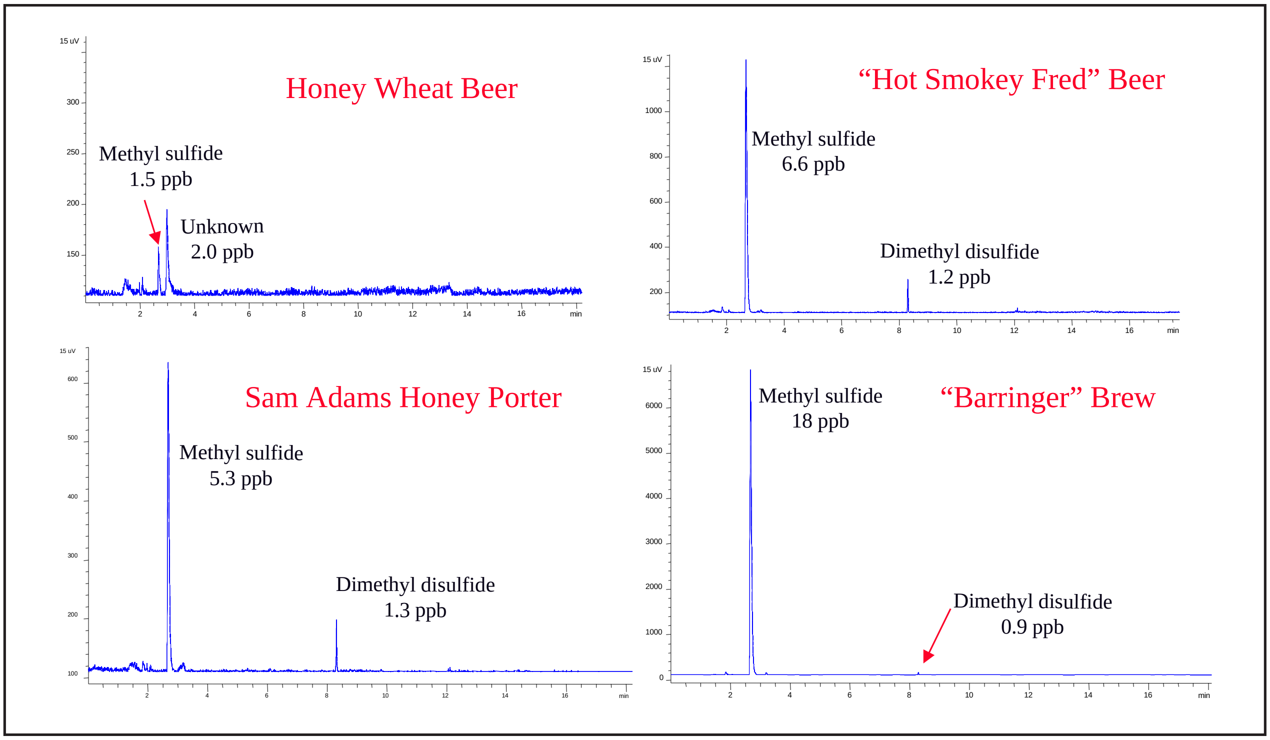


Figure 5. Four Different Beer Samples (Commercial and Home Brewed) Analyzed by Static Headspace with PFPD Detection Showing Concentrations of Light Sulfur Compounds. Concentrations are Reported as ppb Sulfur

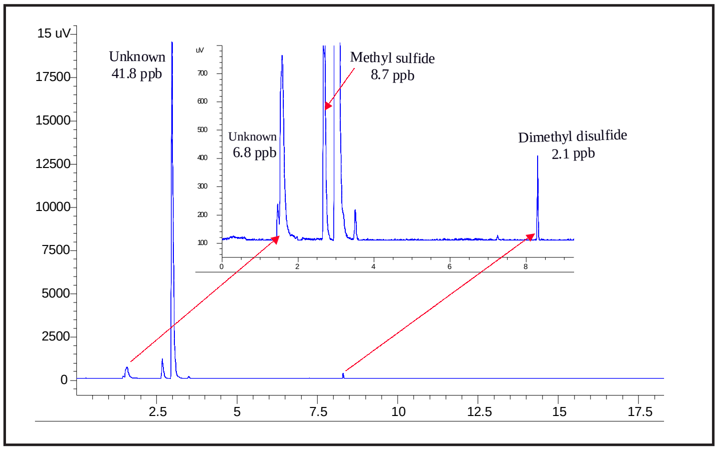


Figure 6. Analysis of Sulfur Compounds in a Dark Red Wine by Static Headspace with PFPD Detection. Concentrations are Reported as ppb Sulfur

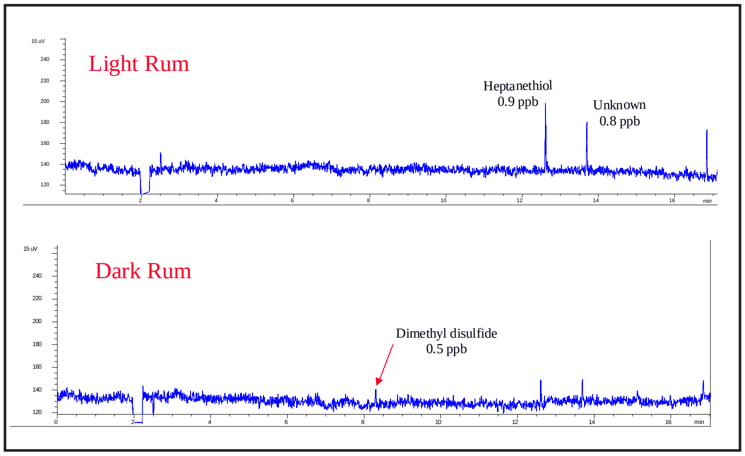


Figure 7. Analysis of Two Different Rum Samples by Static Headspace with PFPD Detection. Concentrations are Reported as ppb Sulfur

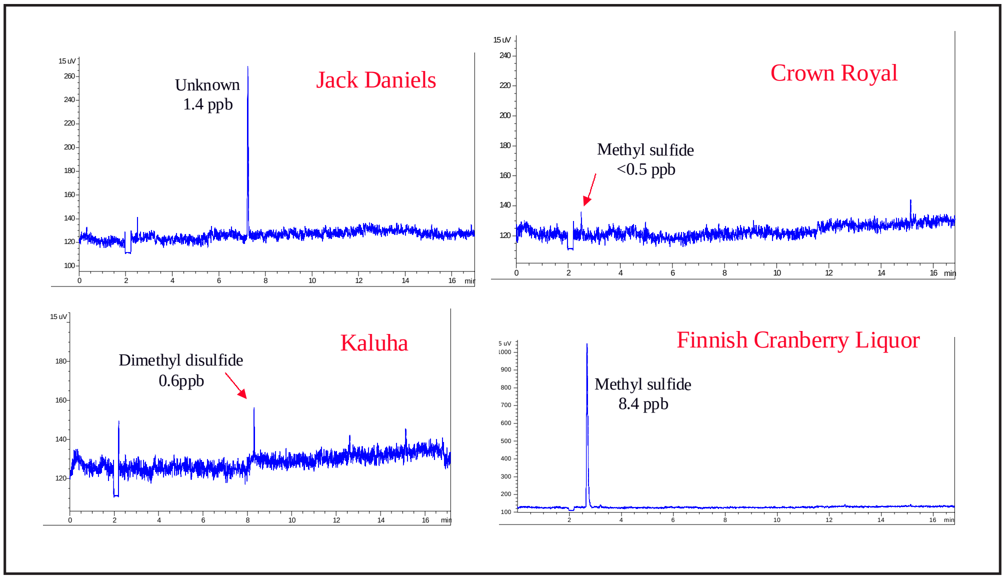


Figure 8. Analysis of Four Different Liquor Samples by Static Headspace with PFPD Detection. Concentrations are Reported as ppb Sulfur

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. All of the calculations for this application note were done in the quadratic mode. 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 can be used to linearize the quadratic response signal associated with sulfur. An example of linearization is shown in Figure 9. For this calibration of three volatile sulfur compounds, the square root function was enabled and both the quadratic and linearized signal responses were recorded. Both calibration curves are shown.

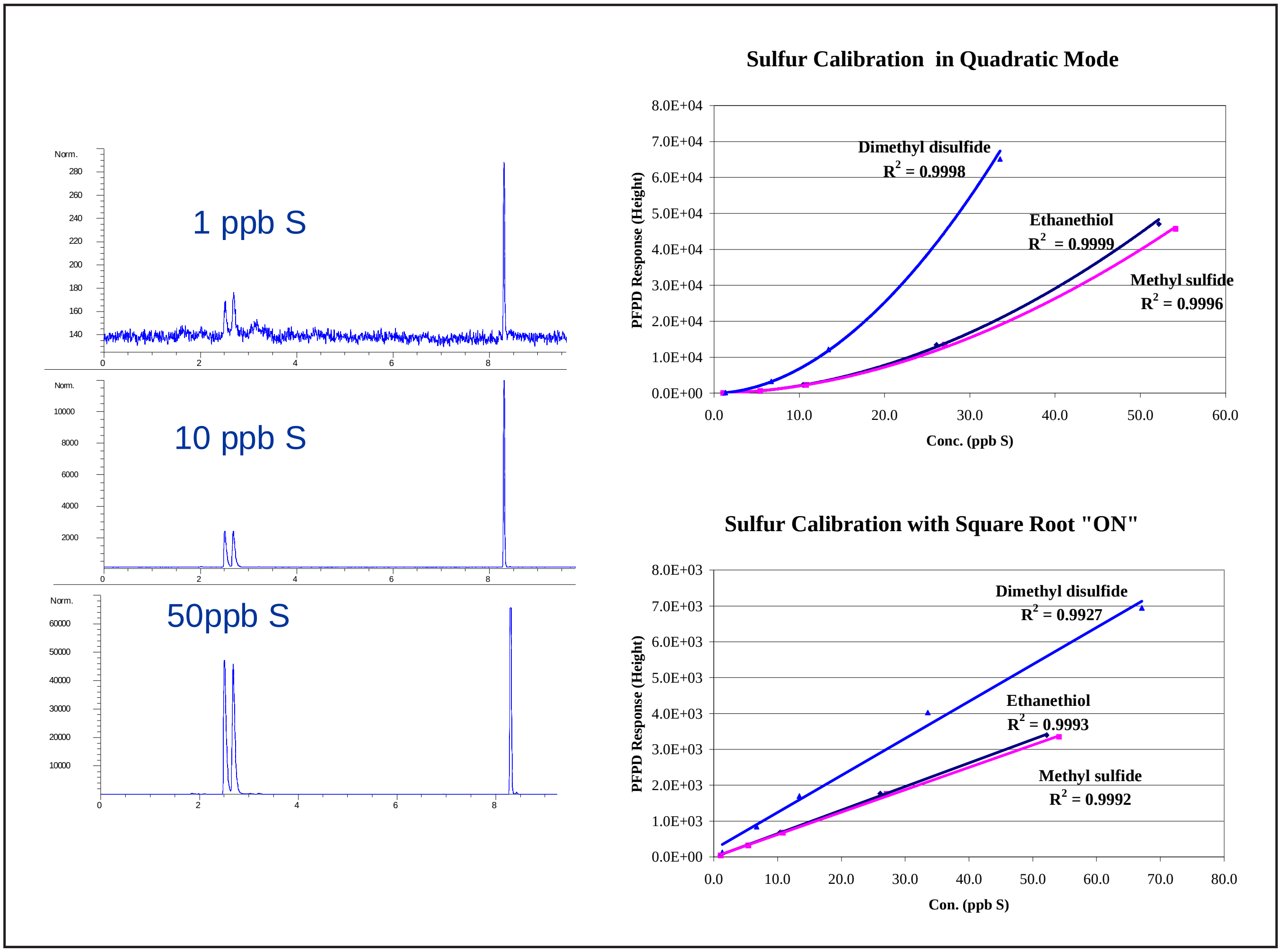
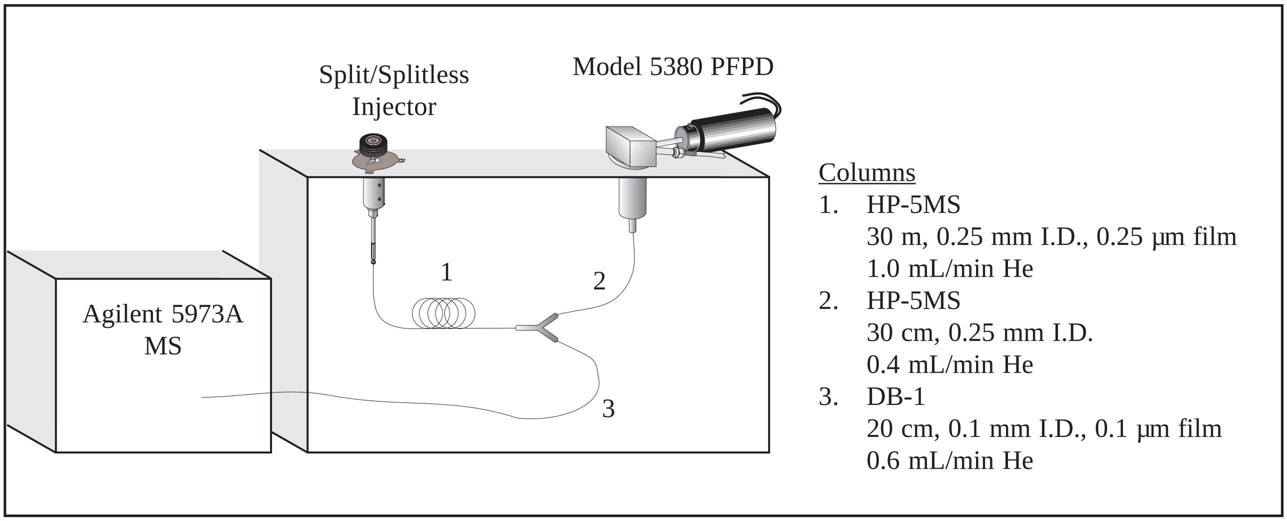


Figure 9. Calibration for 1 ppb to 50 ppb Sulfur in a Three-Component Standard Using Static Headspace with PFPD. R^2 Values Using Peak Areas Ranged from 0.9996 to 0.9999. R^2 Values Using Peak Heights Ranged from 0.9927 to 0.9993

Normally, when the peak areas from a linearized signal are used to calibrate and quantitate sulfur compounds, the measured response is equimolar. This effect is the result of the constant temperature and gas flow conditions within the PFPD combustor and the flame chemistry and characteristic emission profile of the sulfur dimer. However, when using static headspace as the sample introduction technique, the concentration of each compound in the headspace (and thus the concentration (mass) transferred to the GC) is dependent on the vapor pressure at a given temperature. Since this vapor pressure may be quite different for different compounds, the PFPD equimolar response effect may not be observed when using the static headspace autosampler technique.

Sulfur Detection by Tandem PFPD/MS

Using the MS in tandem with the PFPD provides several additional advantages not realized by using either detector alone. The extensive cleanup sometimes necessary with complex matrices can often be eliminated. By running the PFPD in tandem (parallel) with the MS, the PFPD provides a very precise retention time (RT) marking for finding the “sulfur needle in the haystack” of the total ion chromatogram (TIC). Additionally, the PFPD can act as a confirmation detector in complicated analyses. A diagram of the instrumentation used to demonstrate these advantages is shown in Figure 10.



Columns
1. HP-5MS
   30 m, 0.25 mm I.D., 0.25 µm film
   1.0 mL/min He
2. HP-5MS
   30 cm, 0.25 mm I.D.
   0.4 mL/min He
3. DB-1
   20 cm, 0.1 mm I.D., 0.1 µm film
   0.6 mL/min He

Figure 10. PFPD/MS Configuration

A sample of brewed coffee was extracted by simply dropping a Twister stir bar (SPSE) in a vial with the coffee and stirred for approximately two hours. The stir bar is then thermally desorbed onto the GC column and analyzed with the tandem PFPD/MS. The resulting chromatogram is shown in Figure 11. Much of the characteristic flavor and aroma of coffee is the result of a complex mix of sulfur compounds present in the matrix. Identification of specific sulfur compounds in the PFPD sulfur selective chromatogram makes accurate location within the more complicated MS TIC possible. (See Figure 12.) In this way, using the PFPD can be used as a confirmation tool for complex GC/MS analyses and a clear identifier of low-level sulfur peaks in a complex chromatogram. Since mass spectrometers do not have a characteristic ion specific for sulfur, the PFPD adds significant information to assist the analyst in solving difficult analytical challenges when dealing with low-level sulfur analyses.

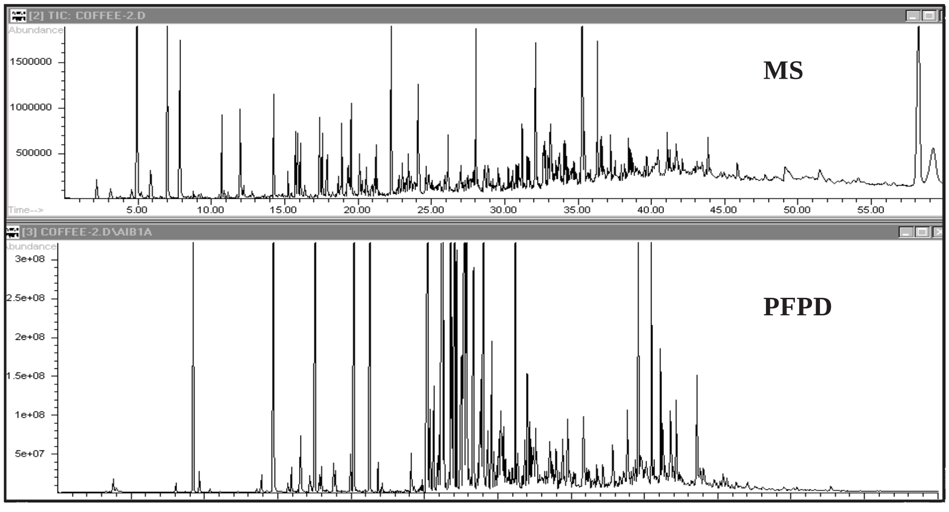
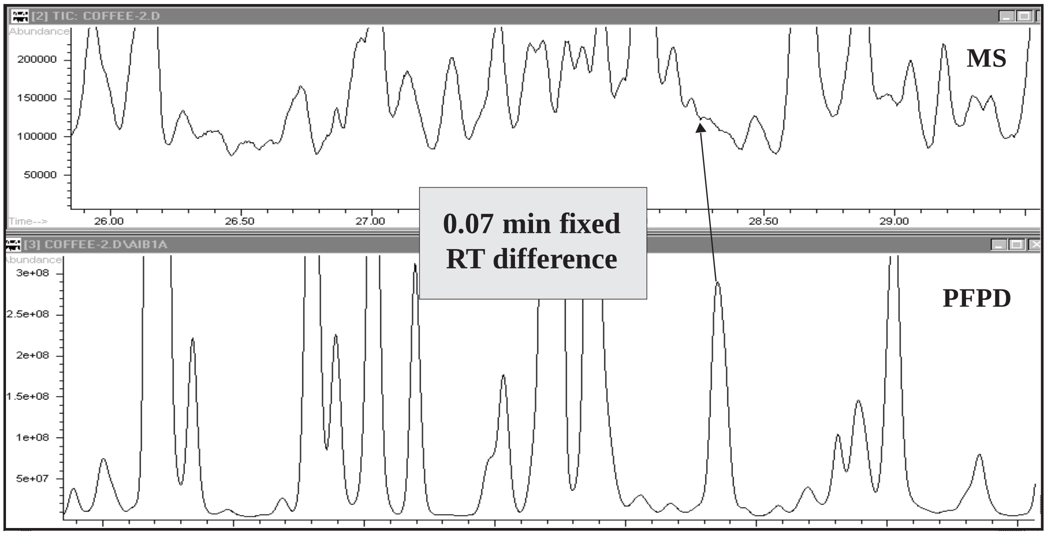


Figure 11. Brewed Coffee by PFPD/MS



0.07 min fixed RT difference

Figure 12. Expanded View of PFPD/MS Chromatogram of Brewed Coffee Showing Identification of Specific Sulfur Compounds in a Complex Total Ion Chromatogram

Conclusions

When coupled with the static headspace autosampler, the PFPD offers good sensitivity for analysis of low-level sulfur compounds in beverage samples. Overall system performance was excellent even at very low ppb levels, with reproducibility (measured as %RSD) of the detector response below 5% for most volatile sulfur analytes. Calibration and quantitation can be done in either the quadratic or linear mode with equally outstanding results. For complex matrices, using the tandem PFPD/MS detectors provides an additional measure of confirmation not available using either detector alone.

Acknowledgements

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