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Detection of Low-Level Sulfur Compounds in Spearmint Oil Using the Pulsed Flame Photometric Detector (PFPD)

Introduction

Two species of spearmint (*mentha spicata* and *mentha gracilis*) are cultivated in the United States. In 2008, 1.09 million kilograms of spearmint oil were produced in the U.S.[1] Forty-five percent of the mint oil produced in the U.S. is used to flavor chewing gum. One 55-gallon drum of mint oil can flavor 5,200,000 sticks of gum or 400,000 tubes of toothpaste.[2]

The principal chemical components in spearmint oil are α-pinene, β-pinene, carvone, 1,8-cineole, linalool, limonene, myrcene, caryophyllene, and menthol.

This study was undertaken to determine if a Pulsed Flame Photometric Detector (PFPD) could be used to find low-level sulfur compounds in spearmint oil. Sulfur compounds impart undesirable odors to essential oils used in flavor and fragrance products. Volatile sulfur compounds have extremely low olfactory thresholds. Detecting, identifying, and eliminating sulfur compounds is an important aspect of flavor and fragrance work.

Associating sulfur compounds by their retention times, with the peaks of hydrocarbon compounds measured by an FID detector provides both qualitative and %total measurements normally used in flavor and fragrance labs.

The objectives of the experimental work were:

- Associate the PFPD carbon element response with the FID detector output for chromatographic verification of the peaks obtained by both techniques
- Use the carbon element to find the sulfur species in the sample by vaporization temperature
- Isolate sulfur species and sulfur containing compounds from the HC compounds by retention times
- Find the area % for all C and S elements and demonstrate the C element percentages are recognizably similar
- Provide an area % for the sulfur species
- Detect the lowest possible sulfur value that can be confirmed and show results

The carbon chromatograms obtained from PFPD and FID detectors are different because the PFPD is an equimolar photometric detector, while the FID responds to and measures all broken hydrocarbon bonds. The combustion cycle occurring in the PFPD breaks down molecules into

atomic species. The number of carbon atoms present determines the resulting response factor.

Selective Detection of Sulfur Heteroatoms with the PFPD

The OI Analytical 5380 PFPD (Figure 1) provides two independent channels of digitized output data. The integration gate for each data channel is set by using WinPulse software to specify the start and stop times of each gate within the PFPD's 25-msec time domain.[3]

Figure 1. OI Analytical 5380 Pulsed Flame Photometric Detector

The emission times of hydrocarbons and heteroatoms often overlap. Figure 2 shows the emission profiles of hydrocarbon, phosphorus, and sulfur. Hydrocarbon emission exhibits little delay, phosphorus emission exhibits a slight delay, and the sulfur emission occurs over a significantly delayed time period. By analyzing a specific time slice of the emitted radiation, the selectivity of the detector can be enhanced significantly. For example, if the time slice from 10-24 milliseconds is analyzed, the majority of the emission is from sulfur with only a small contribution from phosphorus and no hydrocarbon contribution.

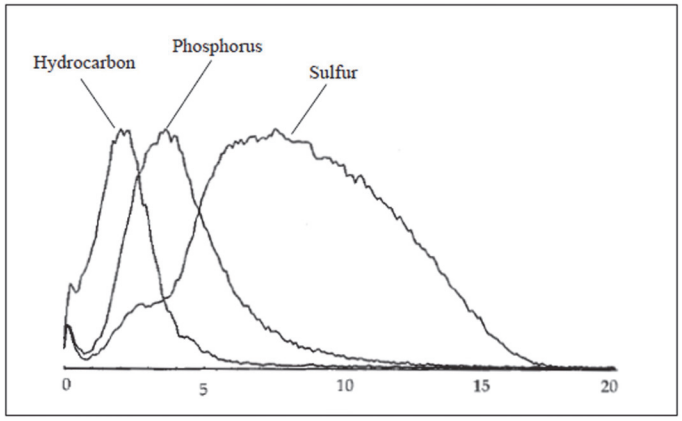


Figure 2. Hydrocarbon, Phosphorus, and Sulfur Emissions in the PFPD

Experimental

Instrumentation used in this study was an Agilent 7890A GC equipped with an OI Analytical 5380 Pulsed Flame Photometric Detector (PFPD) shown in Figure 1 for detection of both Carbon and Sulfur species. Agilent Open Lab Software was employed for acquisition of C and S data into file formats for reports shown. OI Analytical WinPulse Software was used to setup the analysis for C and S species (setting the RF between these two

compounds types allowed display of widely different concentrations on the same output result). WinView 32 software was used to check each peak for the emission timing and characterize it as C and S with the filter and setup used. Details of instrument configuration and operating conditions for PFPD analyses are shown in Table 1. A 3 mm I.D. combustor was used because it quickens the S fluorescence time and provides the highest intensity at 6-16 ms.

Two different samples of neat spearmint oil were tested. The identity of sulfur compounds in the sample was not known, only suspected.

Table 1. Instrument Operating Conditions Used for PFPD Analyses of Spearmint Oil

Gas Chromatograph	Agilent 7890A
Column	DBWAX 30m x 0.25mm ID x 0.5µm
Carrier Gas	Helium
Injection	Manual 0.2 µL using 5 µL syringe, split 70:1
Oven Program	Initial Temp 40 °C hold 3 minutes Ramp 40 to 100 at 5 °C/min hold zero Ramp 100 to 150 at 10 °C / min hold zero Ramp 150 to 220 at 25 °C / min hold 5 minutes
Carbon Detector	Attn 256; Zero 11500
Sulfur Detector	Attn 4; Zero 14
Detector	Range 10
Combustion Chamber	3 mm I.D.
Data Acquisition	Open Lab Software
Emission Time Analysis	WinView 32 Software

Results and Discussion

The peaks were very large and saturated the detector with a 1µL injection and 200:1 split ratio. A 0.1µL injection 70:1 split ratio was found to give good low level results without saturating the C element trace. WinView 32 software was used to compare the PFPD C element trace to the FID HC trace.

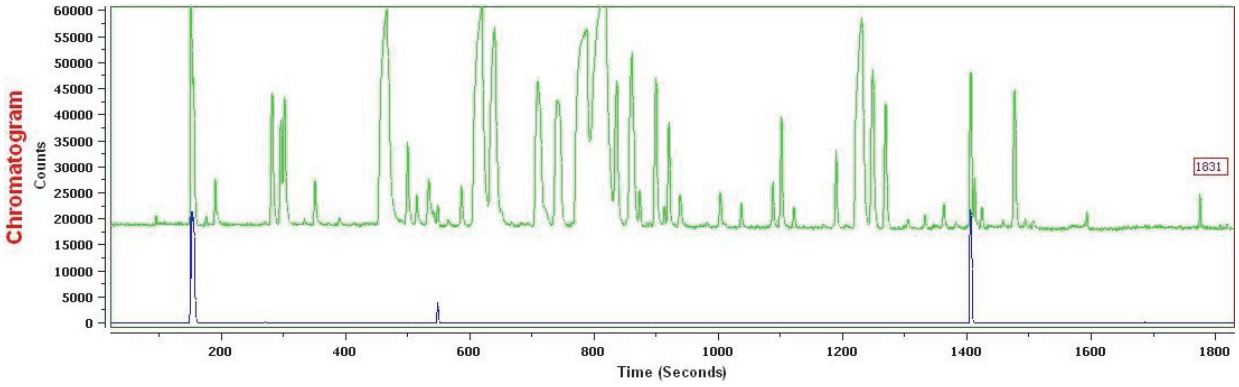


Figure 3. PFPD C and S Element Traces of a Spearmint Oil Sample

The C element trace is shown in gray and sulfur trace underneath it in black arranged on the same X time scale in Figure 3. Is there sulfur in this sample? The short answer is most probably, yes, from this first glance data. More complete peak analysis is required to be certain.

The carbon channel chromatogram in Figure 4 shows peak separation and the 65 peaks that could be labeled with retention times using Open Lab software.

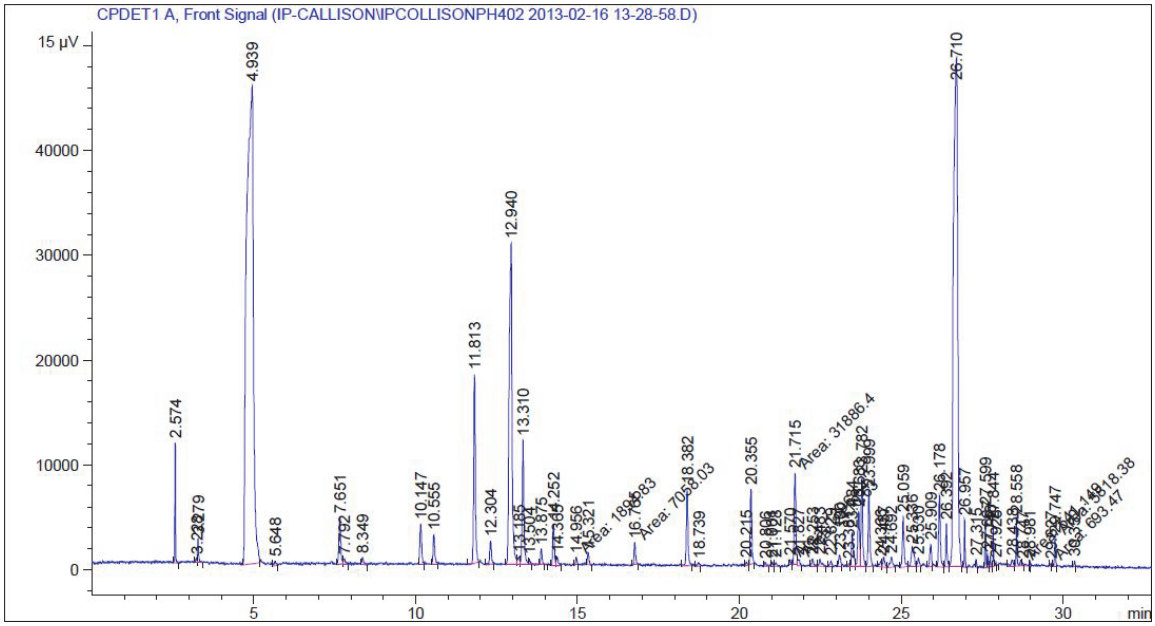


Figure 4. PFPD Carbon Channel Chromatogram of Spearmint Oil from Open Lab Showing 65 Peaks with Labeled Retention Times

The %area report in Figure 5 shows the PFPD chromatographic response factors for major HC peaks expected for spearmint oil. The ratios in the rectangles matched well with expected C percentages from the FID chromatogram, as well as the total response.

Signal 1: CPDET1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [15 µV*s]	Height [15 µV]	Area %
1	2.574	BB S	0.0237	1.75106e4	1.15371e4	0.96885
2	3.228	BV	0.0305	844.92999	402.87482	0.04675
3	3.279	VB	0.0396	5886.87109	2247.63135	0.32572
4	4.939	VV S	0.1689	6.25342e5	4.56245e4	34.59995
5	5.648	BB	0.0570	1798.51697	419.95410	0.09951
6	7.651	BV	0.0606	1.78984e4	4478.30908	0.99031
7	7.792	VB	0.0572	2456.71167	528.37164	0.13593
8	8.349	BB	0.0638	2804.64063	644.50769	0.15518
9	10.147	BB	0.0591	1.46899e4	3797.95020	0.81279
10	10.555	BV	0.0573	1.08053e4	2846.11353	0.59786
11	11.813	VB S	0.0529	6.32471e4	1.80451e4	3.49944
12	12.304	BB	0.0542	8098.33789	2237.95337	0.44808
13	12.940	VV S	0.0977	1.85865e5	3.06700e4	10.28385
14	13.185	VV S	0.0676	3152.74561	777.20422	0.17444
15	13.310	VB S	0.0637	4.54028e4	1.18862e4	2.51212
16	13.504	BV T	0.0354	1122.22742	430.35678	0.06209
17	13.875	VB T	0.0475	4693.57227	1461.52734	0.25969
18	14.252	BV	0.0517	1.32893e4	3902.07568	0.73530
19	14.365	VB	0.0532	3146.02808	795.08417	0.17407
20	14.956	MM	0.0466	1894.82629	677.33234	0.10484
21	15.321	BB	0.0468	3880.31860	1228.86462	0.21470
22	16.765	MM	0.0563	7058.03369	2091.12134	0.39052
23	18.382	VB S	0.0510	2.45784e4	7361.88916	1.35992
24	18.739	BB	0.0620	1719.85559	364.65042	0.09516
25	20.215	BV	0.0473	1225.41626	363.65704	0.06780
26	20.355	VB S	0.0507	2.37366e4	7160.45117	1.31334
27	20.806	BB	0.0529	1461.03162	355.99213	0.08084
28	21.001	BB	0.0366	1035.55591	394.28934	0.05730
29	21.128	BB	0.0498	1513.09082	385.84625	0.08372
30	21.570	MM	0.0485	1396.63538	479.65015	0.07728
31	21.715	MM	0.0604	3.18864e4	8791.59180	1.76427
32	21.827	MM	0.0713	2410.63403	563.16064	0.13338
33	22.253	BB	0.0558	2769.69995	690.85400	0.15325
34	22.483	BB	0.0708	3570.02686	663.08478	0.19753
35	22.829	BB	0.0509	2567.67114	638.82104	0.14207
36	23.100	BV	0.0536	4850.82520	1140.18054	0.26839
37	23.381	BV	0.0496	1308.97107	335.14520	0.07242
38	23.484	VV	0.0592	1.38049e4	3275.33228	0.76382
39	23.683	VV	0.0608	2.05545e4	5358.15234	1.13727
40	23.782	VV S	0.0557	3.12433e4	8540.24121	1.72868
41	23.999	VB S	0.0557	2.64607e4	7229.79785	1.46406
42	24.366	BV	0.0580	2566.41846	544.35883	0.14200
43	24.457	VB	0.0603	4034.60156	936.70361	0.22323
44	24.692	BB	0.0712	5337.10889	955.54785	0.29530
45	25.059	BB	0.0586	1.87392e4	4795.39648	1.03683
46	25.336	BB	0.0795	1.09938e4	1899.97729	0.60829
47	25.530	BB	0.0536	3054.62915	781.12903	0.16901
48	25.909	BV	0.0531	7836.55371	2172.03467	0.43359
49	26.178	BB S	0.0550	2.46258e4	6833.62256	1.36254
50	26.392	BV S	0.0406	1.10467e4	4082.63989	0.61121
51	26.710	VB S	0.1197	4.27873e5	4.86218e4	23.67405
52	26.957	BB S	0.0293	8715.13086	4558.69482	0.48221
53	27.315	BB	0.0254	1183.26074	712.35187	0.06547
54	27.599	VV S	0.0262	9754.91504	5641.22949	0.53974
55	27.667	BV T	0.0241	2107.86914	1288.20361	0.11663
56	27.748	VV T	0.0314	2247.87280	1033.94861	0.12437
57	27.844	VB S	0.0239	6213.62061	4053.36597	0.34380
58	27.926	BB X	0.0253	491.91531	332.41833	0.02722
59	28.438	BV	0.0667	2967.80078	560.97180	0.16421
60	28.558	VB S	0.0261	9572.98438	4661.34033	0.52967
61	28.747	MM	0.0385	541.14929	234.45097	0.02994

Figure 5. Percent Area Report of PFPD C Element Response Factors for a Spearmint Oil Sample

The PFPD found nine S element peaks in this run. The PFPD sulfur channel chromatogram showing the retention times of peaks registered as sulfur compounds is shown in Figure 6.

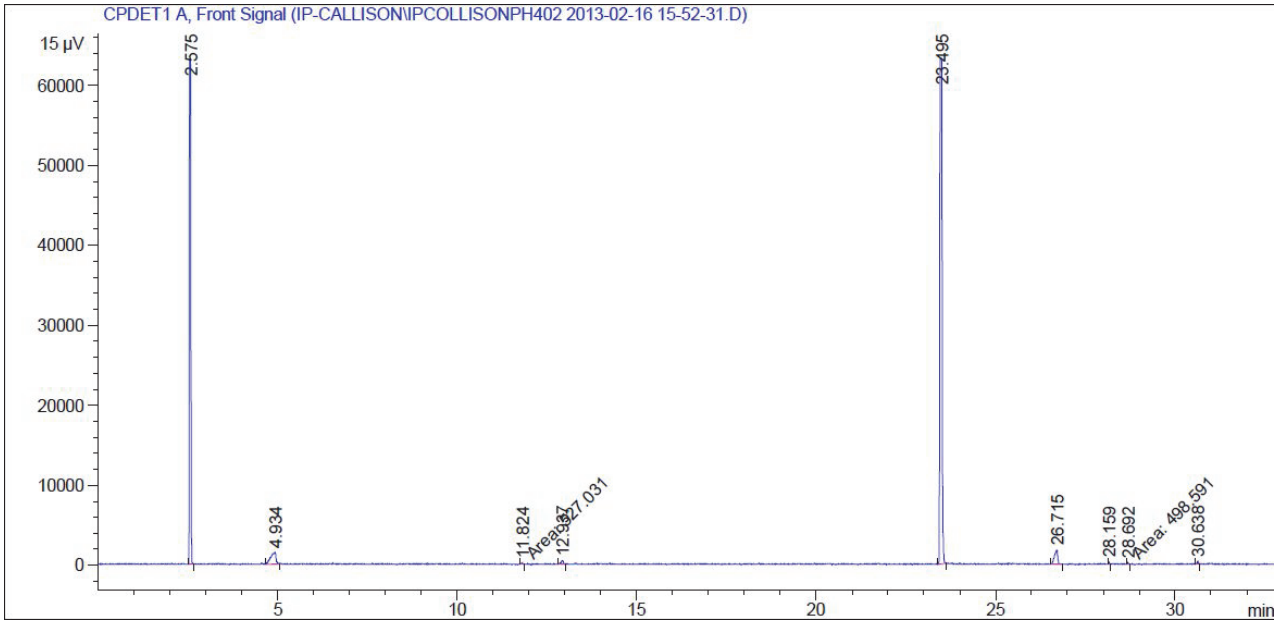


Figure 6. PFPD Sulfur Channel Chromatogram of Spearmint Oil

The next step was to confirm these peaks were not hydrocarbon or phosphorus compounds. Figure 7 shows a sulfur-specific PFPD chromatogram obtained using OpenLab software.

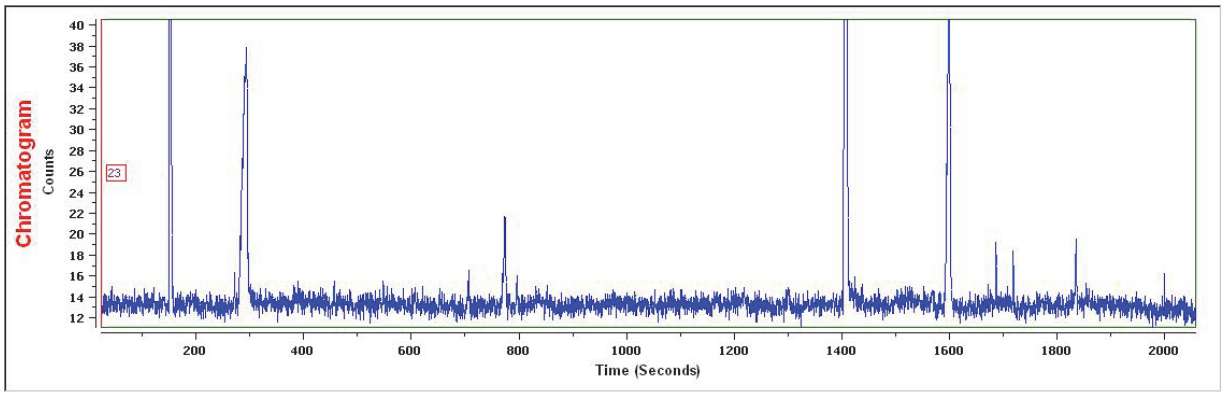
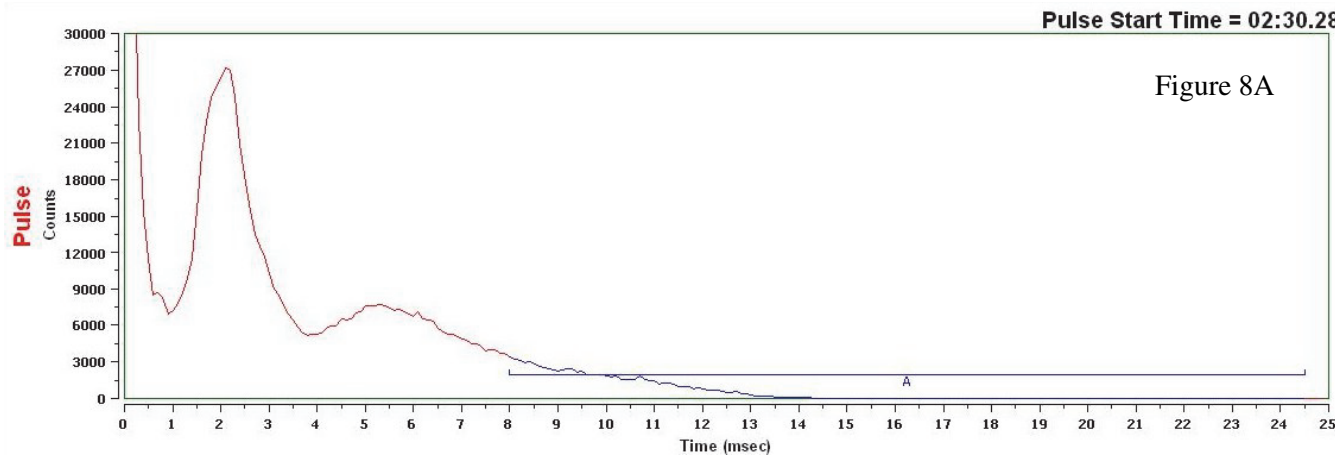
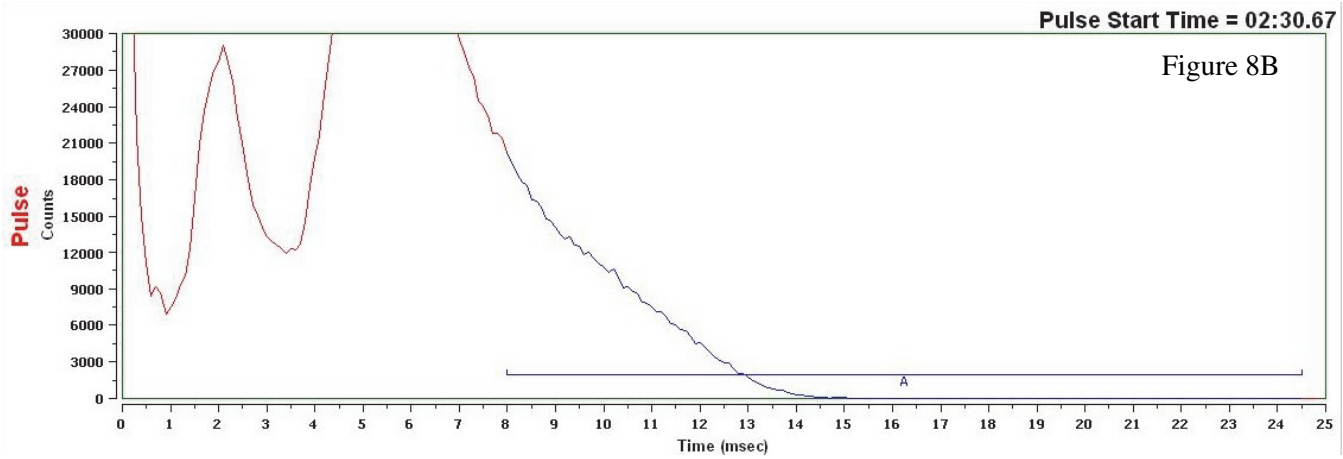


Figure 7. Sulfur-specific PFPD Chromatogram Obtained from OpenLab Software

Figure 8 shows the WinPulse response to a sulfur containing species. This sequence provides an example of how emission times can be used to determine and confirm the validity of a sulfur peak. This sulfur peak begins with a rise at 6ms, then rises sharply without changing the C peak height and widening in the sulfur range out to 16ms (Figure 8 A, B, C). This peak extends past the phosphorus range and does not change the C height confirming it is

a sulfur species.





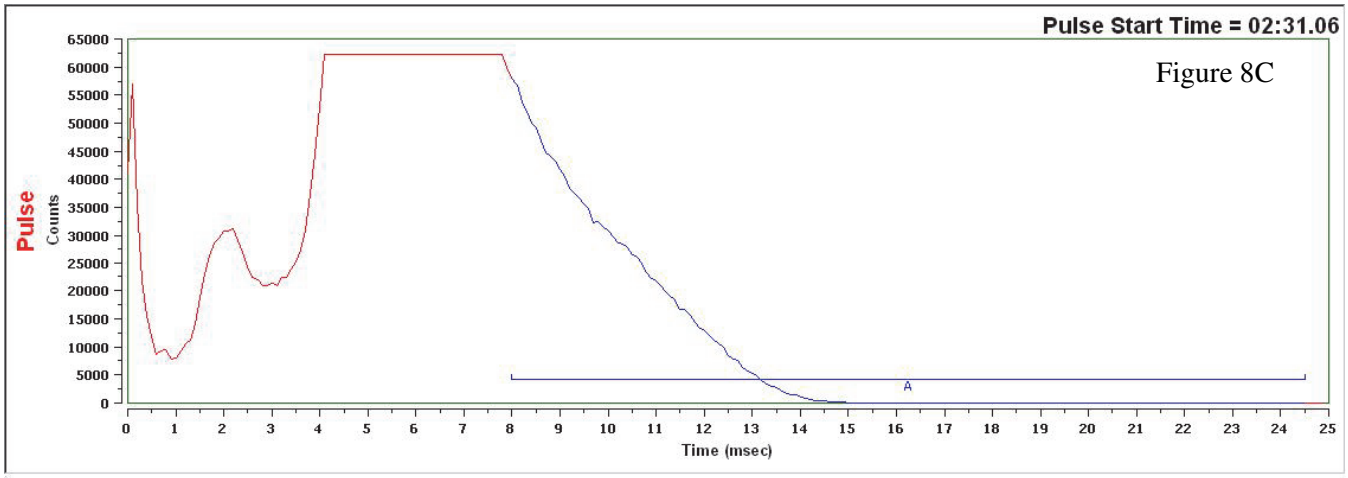


Figure 8. Emission Profile of a Sulfur Peak in a Spearmint Oil Sample

Confirmation of Sulfur Peaks in a Spearmint Oil Sample

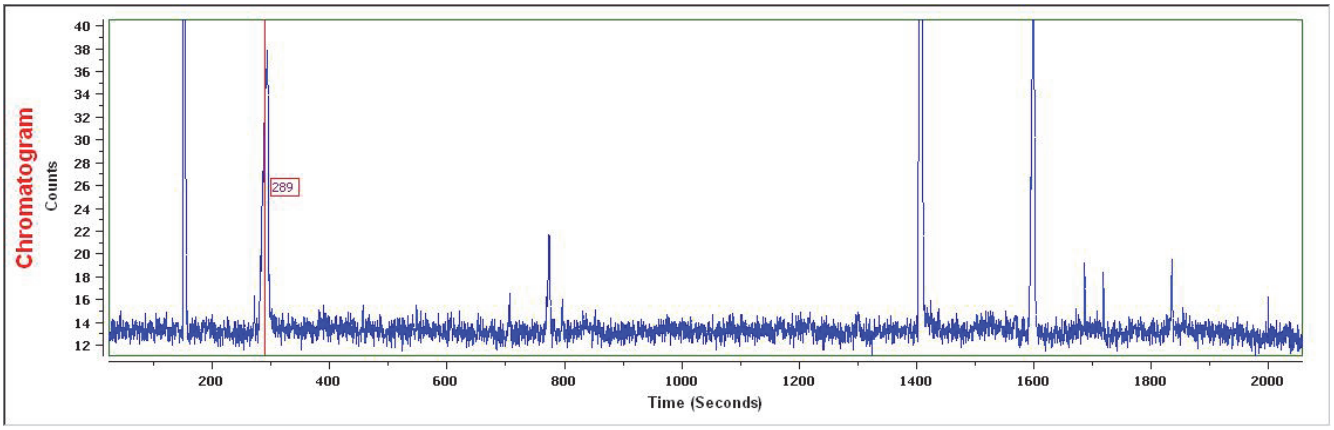


Figure 9. PFPD Sulfur Chromatogram of a Spearmint Oil Sample

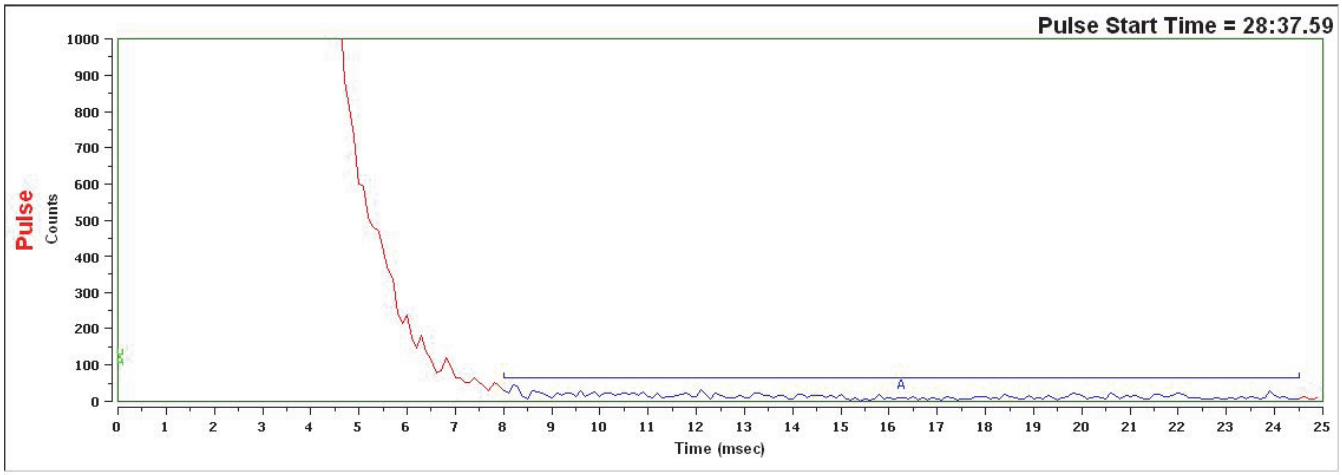


Figure 10. Signal Intensity Change of a Low Level Sulfur Peak in Spearmint Oil

Figures 10 and 11 show the response of low-level sulfur peak compounds using WinPulse software. The intensity change occurring between 8-24.5 msec confirms the presence of sulfur.

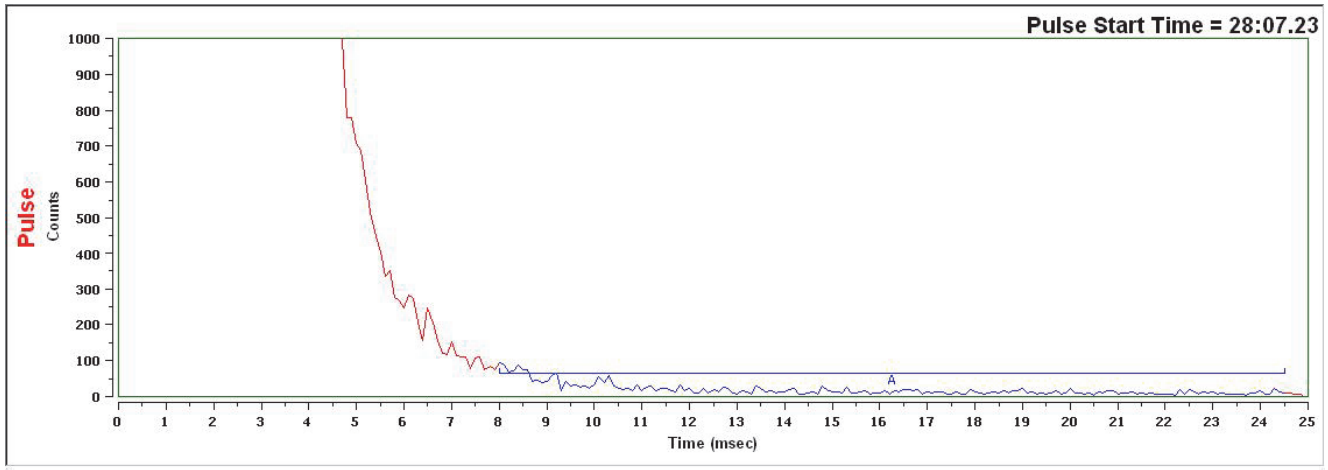


Figure 11. Signal Intensity Change of a Low Level Sulfur Peak in Spearmint Oil

An intensity increase at 8+ msec is a characteristic response for low sulfur containing compounds.

Signal 1: CPDET1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [15 µV*s]	Height [15 µV]	Area %
1	2.575	BB S	0.0476	1.81984e5	6.32469e4	37.97473
2	4.934	BB	0.1307	1.47555e4	1438.49646	3.07903
3	11.824	MM	0.0456	527.03076	192.47113	0.10998
4	12.937	BB	0.0612	2454.14575	492.19568	0.51211
5	23.495	BB S	0.0671	2.65788e5	6.31892e4	55.46209
Peak #	RetTime [min]	Type	Width [min]	Area [15 µV*s]	Height [15 µV]	Area %
6	26.715	BB	0.0828	1.17823e4	1837.53552	2.45862
7	28.159	BB	0.0235	496.28030	331.43024	0.10356
8	28.692	MM	0.0315	498.59137	263.84778	0.10404
9	30.638	BB	0.0355	938.56250	371.43814	0.19585

Figure 12. Response Factor for Nine Peaks in Sulfur Channel Chromatogram of Spearmint Oil

The sulfur channel chromatograms contain nine peaks at retention times reported in Figure 12. There were five confirmed sulfur peaks in this spearmint oil sample.

1. Peak at 2.57 is a high RF sulfur peak with 63246 height counts. This peak contains multiple sulfurs.
2. Peak at 23.49 is also a high RF sulfur peak. 63189.2 height counts. This peak contains multiple sulfurs and has a very high area% of sulfur (over 55% of the total) and 5000:1 signal to noise ratio.
3. Peak at 28.15 is a low RF sulfur peak. The low intensity indicates the peak contains one or two sulfurs.
4. Peak at 28.69 is a low RF sulfur peak. The RF indicates the peak contains only one or two sulfurs and a signal to noise ratio of 10:1.
5. Peak at 30.63 is a low RF sulfur peak. It appears to contain only one or two sulfurs.

Summary and Conclusions

The results of this study demonstrate that low-level sulfur compounds can be detected in spearmint oil using a PFPD detector, dual integration time gates, and comparative carbon peak matching with FID chromatograms. The PFPD C trace matched the FID HC trace for major peaks that have the same number of C atoms as HC bonds.

Five confirmed sulfur peaks were found in each of the spearmint oil samples. The percent total sulfur of the smallest peak that could be detected and confirmed was .00206%.

Response differences between the sulfur peaks show a linear dynamic range of 3+ for S elements. The lowest response for sulfur in the spearmint oil sample was the 28.69 RT peak with a 263.8 height and 10:1 signal to noise ratio.

References

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