

Keywords

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Using the Pulsed Flame Photometric Detector (PFPD) for Low-level Analysis of Organophosphorus Pesticides

Introduction

Organophosphorus (OP) pesticides are among the most widely applied commercial pesticides in the world. Their toxic pesticide residues are present in many agricultural products, often at concentrations in the low parts-per-billion (ppb) range. Food, water, plants, and soils are some of the familiar matrices analyzed for low-level OP pesticide residues.

Two of the most common methods for analyzing OP pesticides include USEPA Method 8141B and Method 1618. Both methods specify using a flame photometric detector (FPD) operated in the phosphorus mode for selective detection of OP pesticides. The OI Analytical Model 5380 Pulsed Flame Photometric Detector (PFPD) shown in Figure 1 has emerged as the phosphorus-selective detector of choice for trace-level OP pesticide analysis. The PFPD uses a pulsed flame, instead of the continuous or static flame of the conventional FPD. The pulsed flame introduces a time-dependent variable to the analysis. As a result, the PFPD outperforms the conventional FPD in detector sensitivity, selectivity for phosphorus with respect to hydrocarbon, lower gas consumption, ease of operation, long-term stability, and dual-element detection capability.

This application note discusses considerations for configuring the PFPD for low-level analysis of OP pesticide residues. Modifying gate settings to extend the calibration range, and using dual gate subtraction to eliminate any residual sulfur interference are discussed. PFPDView software is used as a post-acquisition data processing tool.



Figure 1. OI Analytical Model 5380 Pulsed Flame Photometric Detector (PFPD)

Experimental

The OP pesticides listed in Methods 8141B and 1618 contain phosphorus as the heteroatom, or in many cases, both phosphorus and sulfur. The PFPD can be easily configured for high selectivity of either element with a few simple configuration changes. But when configured for the phosphorus-only mode, the PFPD's sensitivity is approximately ten times lower than in the sulfur mode. The detector can also be configured to detect both phosphorus and sulfur simultaneously using dual-gate subtraction, which can produce mutually selective phosphorus and sulfur chromatograms.

For these experiments, a standard test mixture was selected that included nine pesticides determined by one or both of the USEPA methods. Each OP pesticide contained at least one sulfur and one phosphorus atom so calibration curves in both configurations could be compared. All analyses were conducted using an Agilent® 6890N Gas Chromatograph (GC) configured with a split/splitless injector and an OI Analytical Model 5380 PFPD. Agilent ChemStation® was used to acquire and process the data. Table 1 shows instrument operating conditions.

Table 1. Instrument operating conditions

Parameter	Specification
GC	Agilent 6890N
Inlet	Split/splitless inlet, splitless mode, 4-mm single gooseneck splitless liner, 220 °C, 1-µL injections
Column	Rtx®-5, 30 m x 0.32-mm I.D. x 0.5-µm film
Carrier gas	Helium, 1.1 mL/minute, constant flow mode
Oven program	50 °C (2 minutes), 8 °C/minute to 300 °C (5 minutes)
Detector	Model 5380 PFPD
Board parameters	PMT voltage = 550 Ignitor current = 2.8 Trigger level = 500 Range = 10
Phosphorus mode	3-mm combustor Yellow RG-495 optical filter H ₂ /air combustion gas in approximately equal proportions Phosphorus gate at 4–10 msec (see discussion for other gates)
Sulfur mode	3-mm combustor (using a 2-mm combustor improves sulfur sensitivity, but sacrifices phosphorus sensitivity) Blue BG-12 optical filter H ₂ /air combustion gas, slightly H ₂ -rich Sulfur gate at 6–25 msec, square root ON
P+S dual element mode	3-mm combustor Clear UV-34 optical filter H ₂ /air combustion gas same as phosphorus mode Dual-gate subtraction (see discussion)

Results and Discussion

Phosphorus Mode

Calibration standards were prepared containing all nine compounds at concentrations ranging from 1 to 2,000 ppb. The standards were analyzed using the PFPD configured in the phosphorus mode and a calibration curve was plotted. The chromatogram from analysis of the 100-ppb standard is shown in Figure 2. Table 2 lists the compound names with their chemical formulas, molecular weights, and weight percents of phosphorus and sulfur.

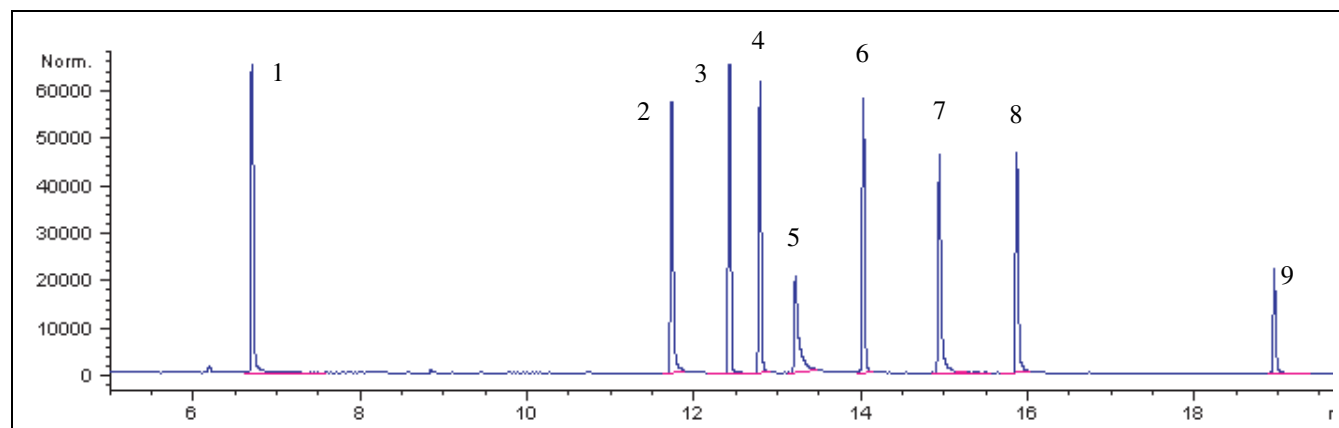


Figure 2. PFPD chromatogram of the 100-ppb calibration standard containing nine of the OP pesticides found in USEPA Methods 8141B and 1618. The PFPD was configured for phosphorus-selective detection with a 3-mm combustor, RG-495 optical filter, and 4–10 msec gate. See Table 2 for compound identification and percent phosphorus content.

Table 2. OP pesticide compounds

No.	Name	CAS No.	Formula	Mol. Weight	Wt. % P	Wt. % S	8141B Analyte	1618 Analyte
1	<i>o,o,o</i> -Trimethylphosphorothioate	152-20-5	C ₃ H ₉ O ₃ PS	156.1	19.8	20.5		
2	Thionazin	297-97-2	C ₈ H ₁₃ N ₂ O ₃ PS	248.2	12.5	12.9	✓	
3	Sulfotepp	3689-24-5	C ₈ H ₂₀ O ₅ P ₂ S ₂	322.3	19.2	19.9	✓	✓
4	Phorate	298-02-2	C ₇ H ₁₇ O ₂ PS ₃	260.4	11.9	36.9	✓	✓
5	Dimethoate	60-51-5	C ₅ H ₁₂ NO ₃ PS ₂	229.3	13.5	27.9	✓	✓
6	Disulfoton	298-04-4	C ₈ H ₁₉ O ₂ PS ₃	274.4	11.3	35.1	✓	✓
7	Methyl parathion	298-00-0	C ₈ H ₁₀ NO ₅ PS	263.2	11.8	12.2	✓	✓
8	Parathion	56-38-2	C ₁₀ H ₁₄ NO ₅ PS	291.3	10.6	11.0		✓
9	Famphur	52-85-7	C ₁₀ H ₁₆ NO ₅ PS ₂	325.3	9.5	19.7	✓	✓

The PFPD is a highly selective detector, and when configured for phosphorus detection, responds only to the phosphorus in the compound. It also has the added advantage of an equimolar response for phosphorus, so it produces the same total response for equivalent amounts of phosphorus regardless of the parent compound. This effect can be observed by comparing peak areas from the chromatogram in Figure 2 to the weight percent phosphorus for each compound. The calculated response factors for the phosphorus in each compound are shown in Table 3. The low 6.1% RSD over the nine response factors is a confirmation of the equimolarity of the phosphorus response. This conclusion is further confirmed by the chromatogram of the 1-ppb standard (Figure 3), which only shows peaks for the two compounds with the highest percent phosphorus content, *o,o,o*-trimethylphosphorothioate and sulfotepp. The phosphorus content for these two peaks was approximately 0.2 ppb (0.2 pg of phosphorus on the column). Note that peak heights cannot be used for this test as the peak width varies for

individual pesticides across the chromatogram.

Table 3. PFPD area counts for the nine OP pesticides shown in Figure 2, with calculated response factors illustrating equimolar response of the PFPD. Each compound was present in the mixture at 100 ppb.

No.	Name	Wt. % P	Area Counts	Response Factor
1	<i>o,o,o</i> -Trimethylphosphorothioate	19.8	46,573	2,352
2	Thionazin	12.5	25,967	2,077
3	Sulfotepp	19.2	43,507	2,266
4	Phorate	11.9	25,798	2,168
5	Dimethoate	13.5	31,832	2,358
6	Disulfoton	11.3	24,336	2,154
7	Methyl parathion	11.8	25,318	2,146
8	Parathion	10.6	23,227	2,191
9	Famphur	9.5	18,385	1,935
Average Response Factor				2,183
RF % RSD				6.1

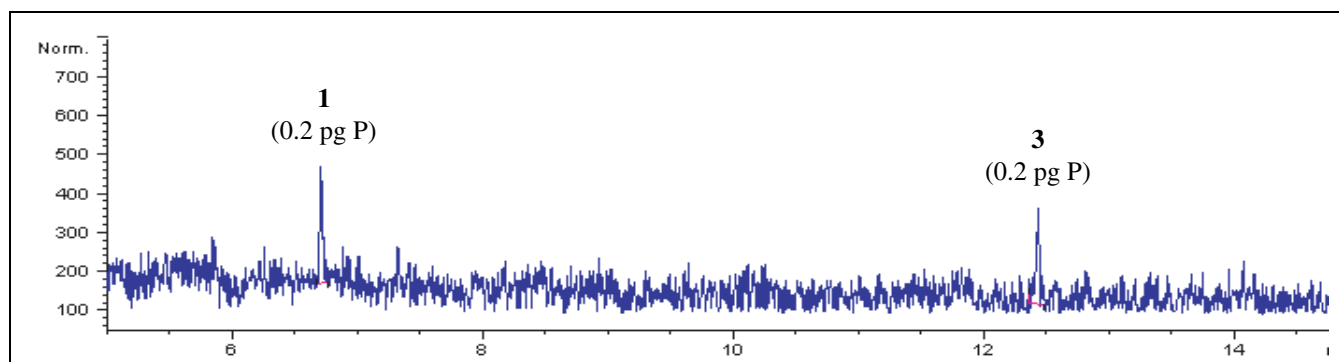


Figure 3. PFPD chromatogram (phosphorus mode) of the 1-ppb calibration standard showing peaks for the two compounds with the highest percent phosphorus content. These two peaks each represent approximately 0.2 pg of phosphorus on the column.

When plotting the PFPD responses for sulfotepp as a function of concentration over the entire calibration range (1 to 2,000 ppb), the calibration curve clearly shows that the linear range of the detector has been exceeded using these conditions (Figure 4). A close examination of the individual phosphorus emissions using PFPDView software makes the reason clear. Figure 5 is a screen from PFPDView showing the overlaid phosphorus emissions from sulfotepp at 50 and 2,000 ppb, using a gate setting of 4–10 msec. As long as the phosphorus emission within the defined gate remains within scale and does not saturate the window, the signal remains linear. In this example, when the gate was set at 4–10 msec, the emission from the 50-ppb sulfotepp peak remained within scale and within the linear range. Once the emission reached the top of the scale, the curve was no longer linear. For sulfotepp, the phosphorus emission begins to saturate the 4–10 msec gate at 100 ppb (approximately 19.2 ppb of phosphorus), producing a linear calibration curve up to 100 ppb with an R^2 value of 0.9971 (Figure 6).

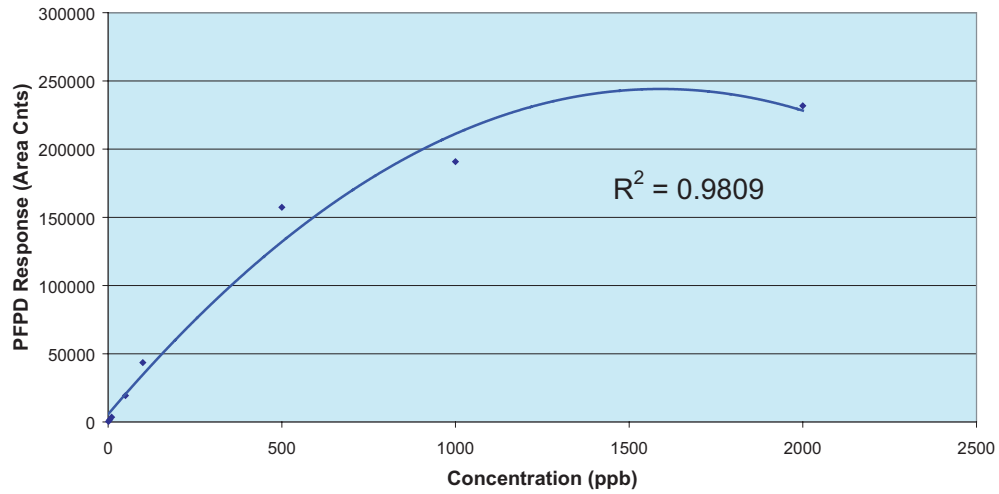


Figure 4. Calibration curve for sulfotepP over the full calibration range (1 to 2,000 ppb) using a 4–10 msec phosphorus gate

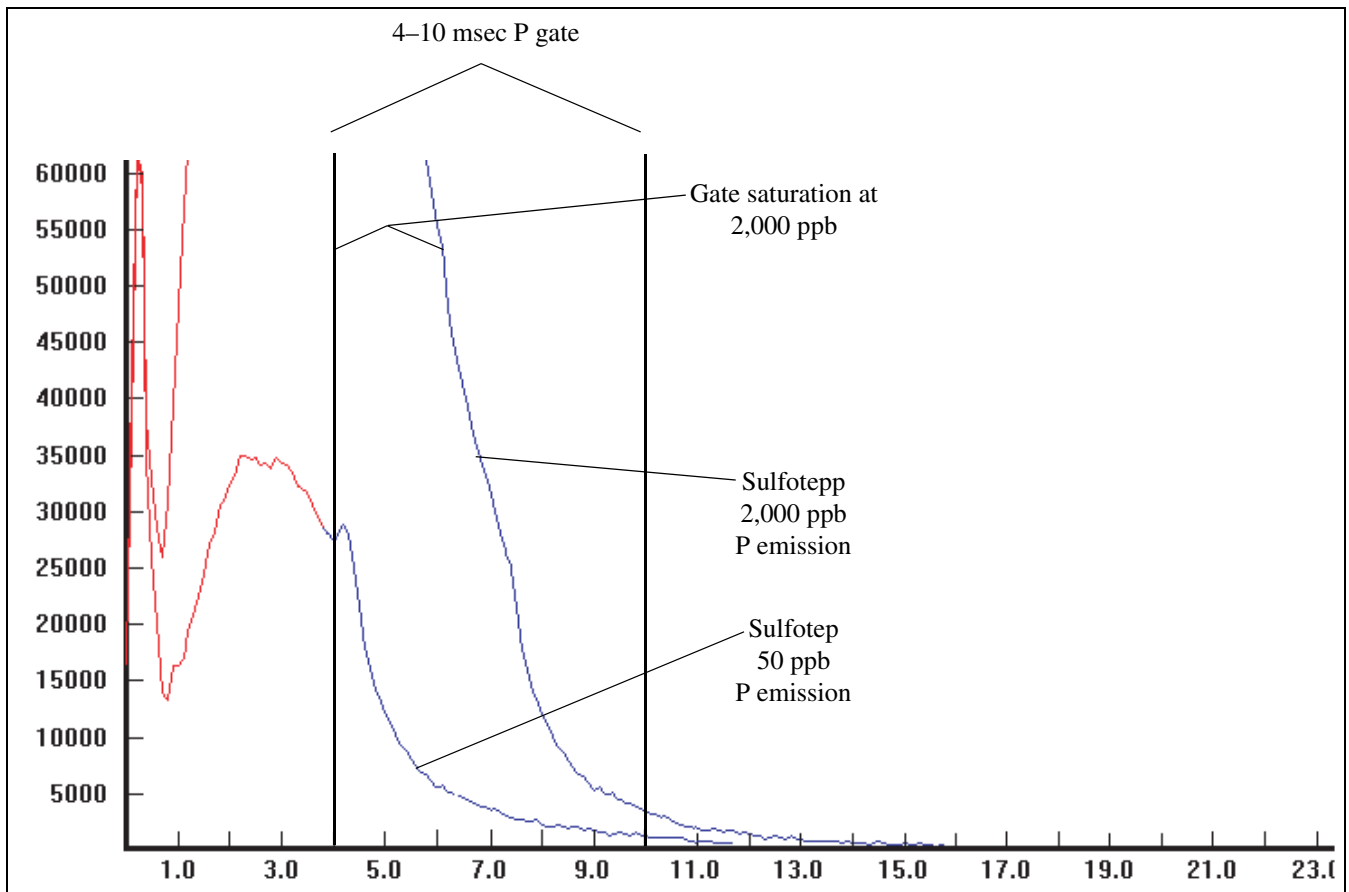


Figure 5. PFPDView screen illustrating overlaid phosphorus emissions from 50 ppb and 2,000 ppb of sulfotepP with a gate setting of 4–10 msec. Note that the 2,000-ppb phosphorus emission is saturated within the 4–10 msec gate, leading to a nonlinear response.

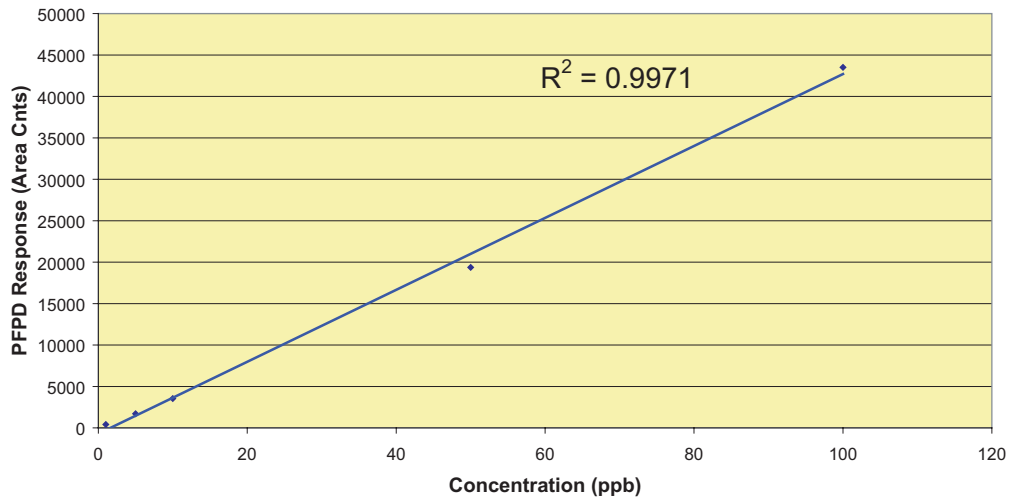


Figure 6. Linear calibration curve for sulfotepp from 1 to 100 ppb using a 4–10 msec phosphorus gate

When necessary, the linear range of the PFPD can be easily extended by simply modifying the gate setting. For sulfotepp, when the gate was changed to 6–10 msec, the phosphorus emission no longer saturated the gate, even at 2,000 ppb. Using the shorter delayed gate setting, the calibration range for sulfotepp was extended to 2,000 ppb, and resulted in R^2 values of 0.9936 for a linear fit or 0.9997 when using a quadratic fit (Figure 7 and Figure 8).

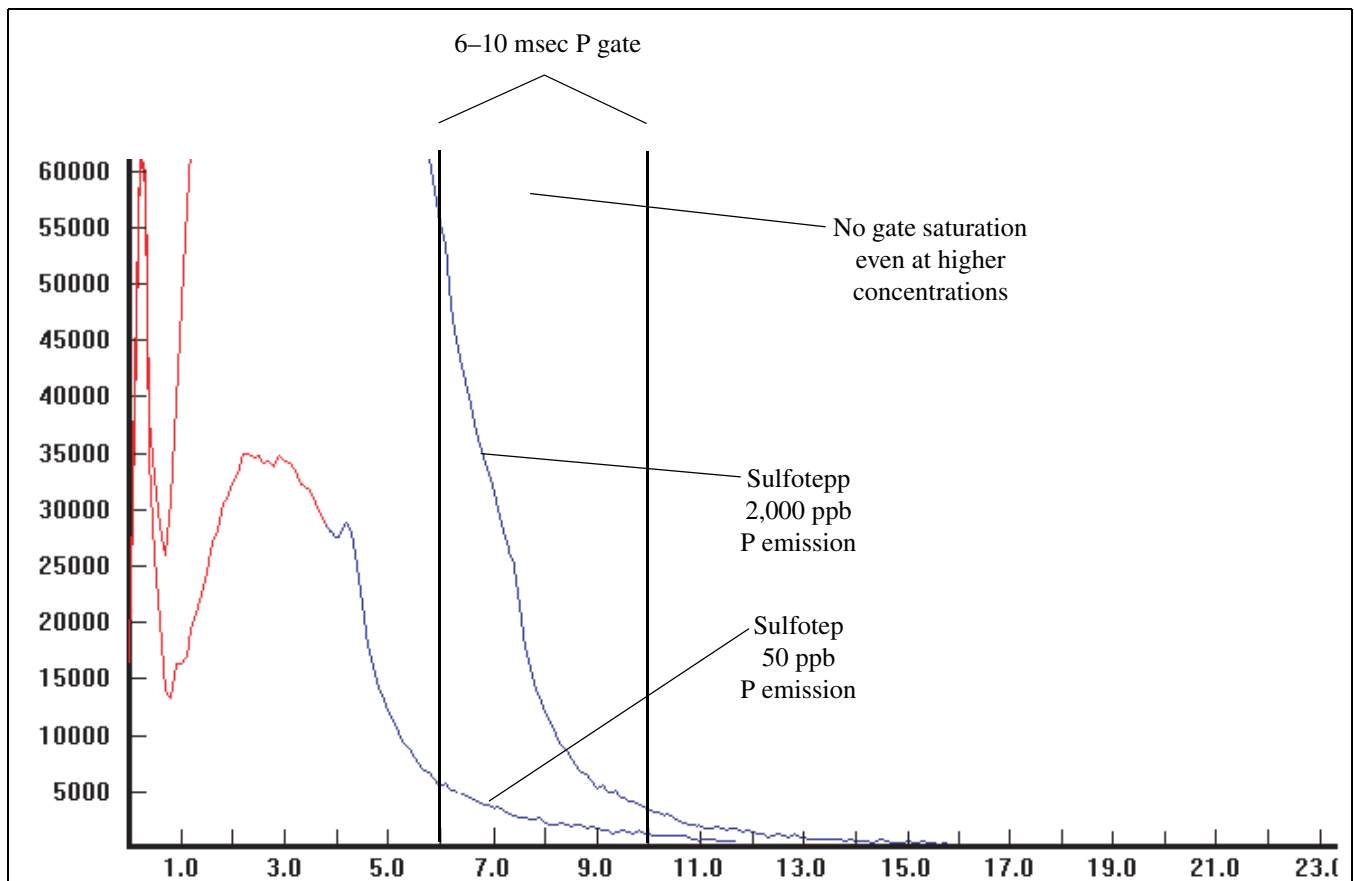


Figure 7. PFPDView screen illustrating overlaid phosphorus emissions from 50 ppb and 2,000 ppb of sulfotepp with a gate setting of 6–10 msec. Note that the 6–10 msec gate is not saturated, even at 2,000 ppb, leading to a linear calibration up to 2,000 ppb.

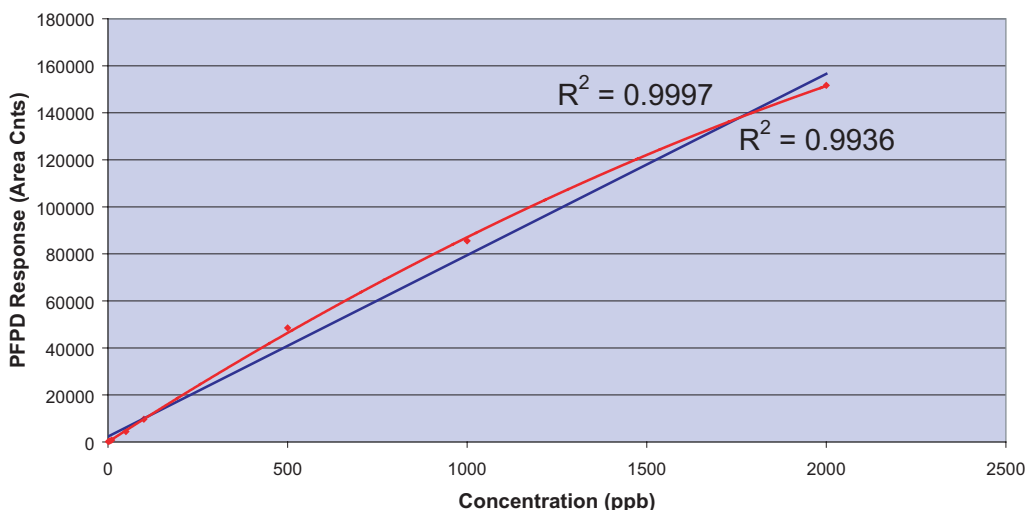


Figure 8. Calibration curve for sulfotep over the full calibration range (1 to 2,000 ppb) using a 6–10 msec phosphorus gate. Either a linear (0.9936) or quadratic (0.9997) curve fit can be used for this calibration.

The one drawback to using the delayed 6–10 msec gate can be a slight loss of sensitivity at the low end of the calibration curve. One technique for mitigating this loss is to configure two separate gates for phosphorus. Since the PFPD controller uses a digital processor, it is possible to output two simultaneous signals and two simultaneous chromatograms by simply defining two different phosphorus gates. For sulfotep, the first signal would be configured with a 4–10 msec gate for the low-level concentrations (up to about 100 ppb), and a second signal would be configured with the delayed 6–10 msec gate for quantitation at the high end of the concentration range. Using this technique, the calibration range can be extended by one full order of magnitude without sacrificing sensitivity at the low end.

Sulfur Mode

The PFPD was reconfigured for selective detection of sulfur by switching to the BG-12 optical filter, changing the gate to 6–25 msec to capture the sulfur emissions, and turning on the square root function to linearize the naturally quadratic sulfur signal. Results from sulfur selective analyses were similar to the phosphorus results; the primary difference was that the PFPD was approximately 10 times less sensitive in the sulfur mode. Figures 9, 10, and 11 illustrate the sulfur results.

In PFPDView (Figure 10), the sulfur emission from phorate at 50 ppb is not saturated and is within the linear range of the 6–25 msec gate. The second emission, phorate at 500 ppb, is just below the saturation point and represents the top of the linear range. The sulfur emission of phorate at 2,000 ppb is completely saturated throughout most of the 6–25 msec gate and is beyond the linear range for the gate shown. In this example, the linear calibration range for phorate was 10 to 500 ppb (Figure 11). The 500-ppb calibration standard represented approximately 185 pg of sulfur on the column and was the saturation point for the sulfur emission.

If desired, the sulfur calibration range can also be extended by defining a second sulfur gate for a portion of the sulfur emission that does not saturate at the higher concentrations. In Figure 10, the second gate at 3–5 msec does not saturate until approximately 1,500 pg sulfur on column, and would extend the linear calibration range by almost one order of magnitude. This is exactly analogous to the phosphorus example previously discussed.

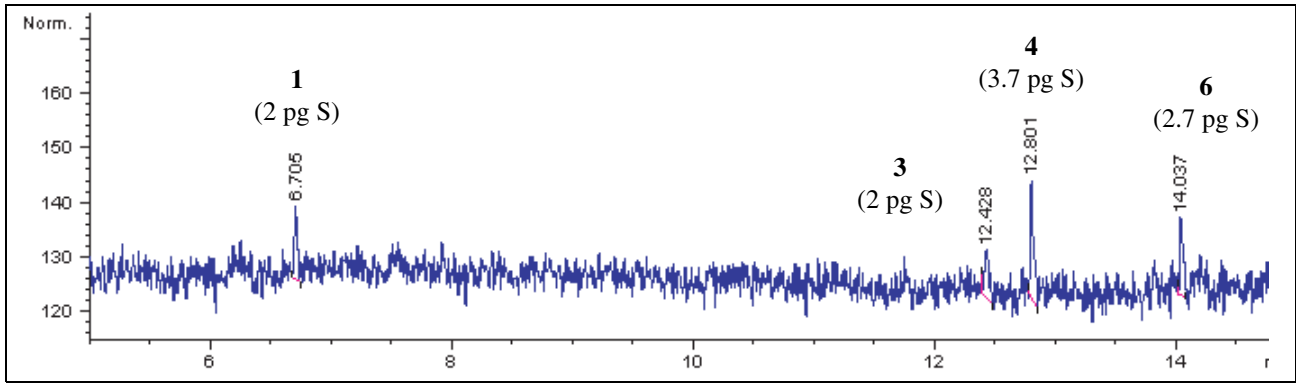


Figure 9. PFPD chromatogram (sulfur mode) of the 10-ppb calibration standard, showing peaks for four of the compounds with the highest sulfur content. The peaks shown here represent approximately 2 to 4 pg of sulfur on the column. See Table 2 for compound identification and sulfur content.

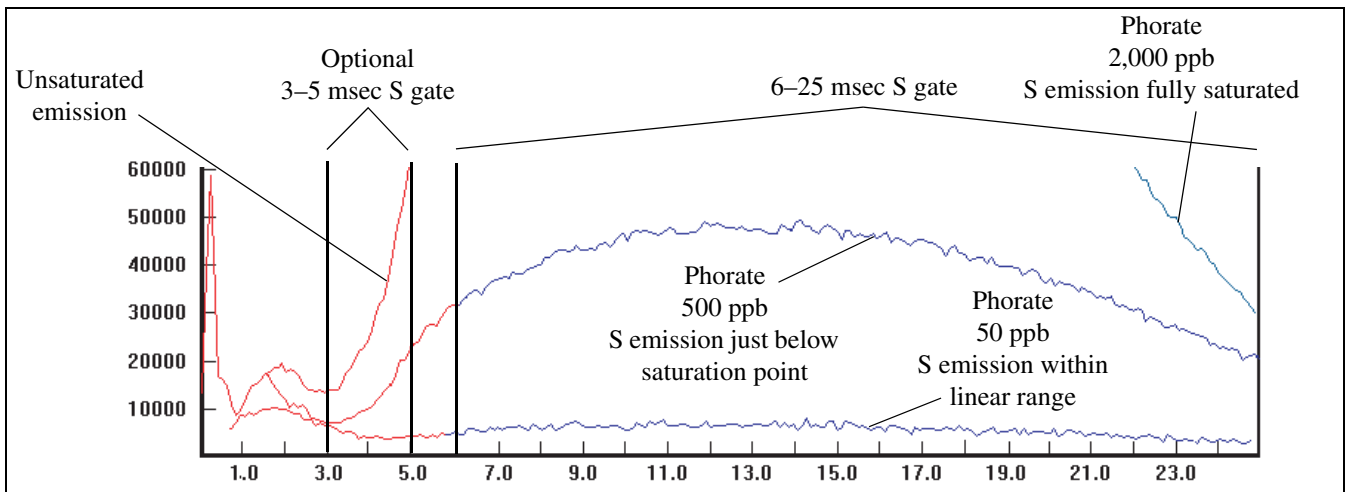


Figure 10. PFPDView screen illustrating overlaid sulfur emissions for phorate at 50 ppb, 500 ppb, and 2,000 ppb, and a gate setting of 6–25 msec. The optional second gate at 3–5 msec can be used to extend the linear calibration range by one full order of magnitude.

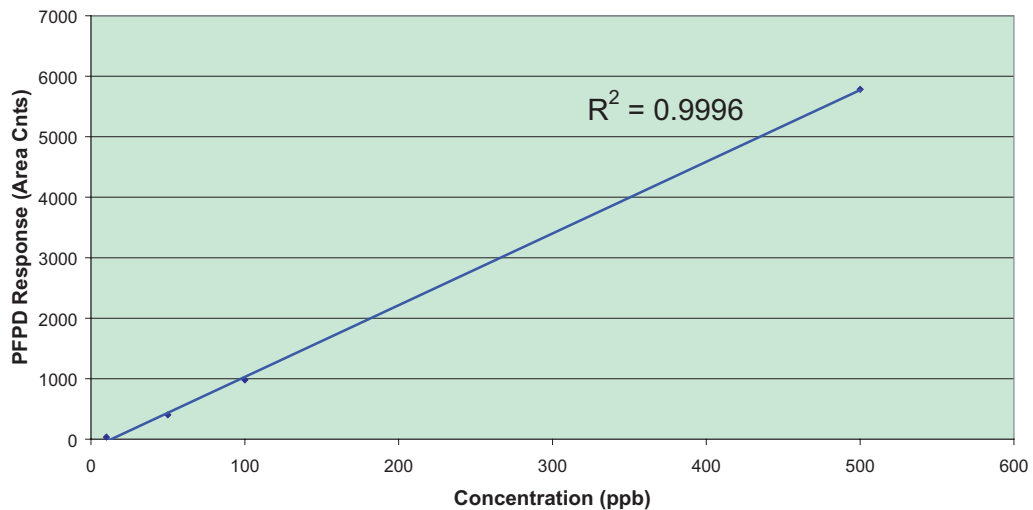


Figure 11. Linear calibration curve for phorate from 10 to 500 ppb using a 6–25 msec sulfur gate

PFPDView Software and Dual Gate Subtraction of Sulfur Interference

Some sample types, particularly agricultural products like onions, garlic, and peppers, may contain very high concentrations of naturally occurring sulfur compounds in the sample extract that have the potential to interfere when the PFPD is configured for selective phosphorus detection. These sulfur compounds have weak spectral emission lines that fall within the transmission curve of the RG-495 optical filter used in phosphorus analyses, and are normally not observed under standard conditions. However, when the sulfur compounds are present at extremely high concentrations, as in the case of some food products, false positive peaks in the phosphorus chromatogram may result.

PFPDView can be used to optimize PFPD parameters, effectively remove the sulfur interference from the chromatogram, and result in phosphorus-only detection. The individual emissions (pulses) from a chromatographic run are saved as a data file (*.dat) using WinPulse. These data files are then imported into PFPDView, which allows the operator to observe the individual emission profiles from any peak in the chromatogram, and identify the heteroatom as either sulfur or phosphorus. The operator can also use PFPDView to modify the PFPD gate parameters and reprocess the original data to determine the effect of new gate settings on the chromatogram without having to rerun the analysis. This is a powerful method development tool that predicts the outcome of PFPD acquisition variables on the chromatogram in a matter of minutes, instead of the hours it would take to run multiple combinations of variables.

To illustrate PFPDView and dual-gate subtraction, the PFPD was configured in the phosphorus selective mode with an RG-495 filter, 3-mm combustor, and 4–10 msec phosphorus gate. A garlic extract with high sulfur content was spiked with four OP pesticides at varying concentrations and analyzed on the PFPD. The result of the original PFPD analysis is shown in the screen from PFPDView (Figure 12). The vertical black cursor in Figure 12A is positioned on the chromatographic peak at approximately pulse 201; the corresponding emission in the top half of the screen clearly identifies this as a sulfur-containing compound, a false positive in this phosphorus chromatogram. Moving the cursor to the peak at pulse 520 (Figure 12B) displays a waveform now dominated by a phosphorus emission and identifies this peak as one of the OP pesticides spiked into the matrix. Since the extract has been spiked with only four phosphorus containing compounds, most of the peaks in the chromatogram are clearly the result of sulfur interference.

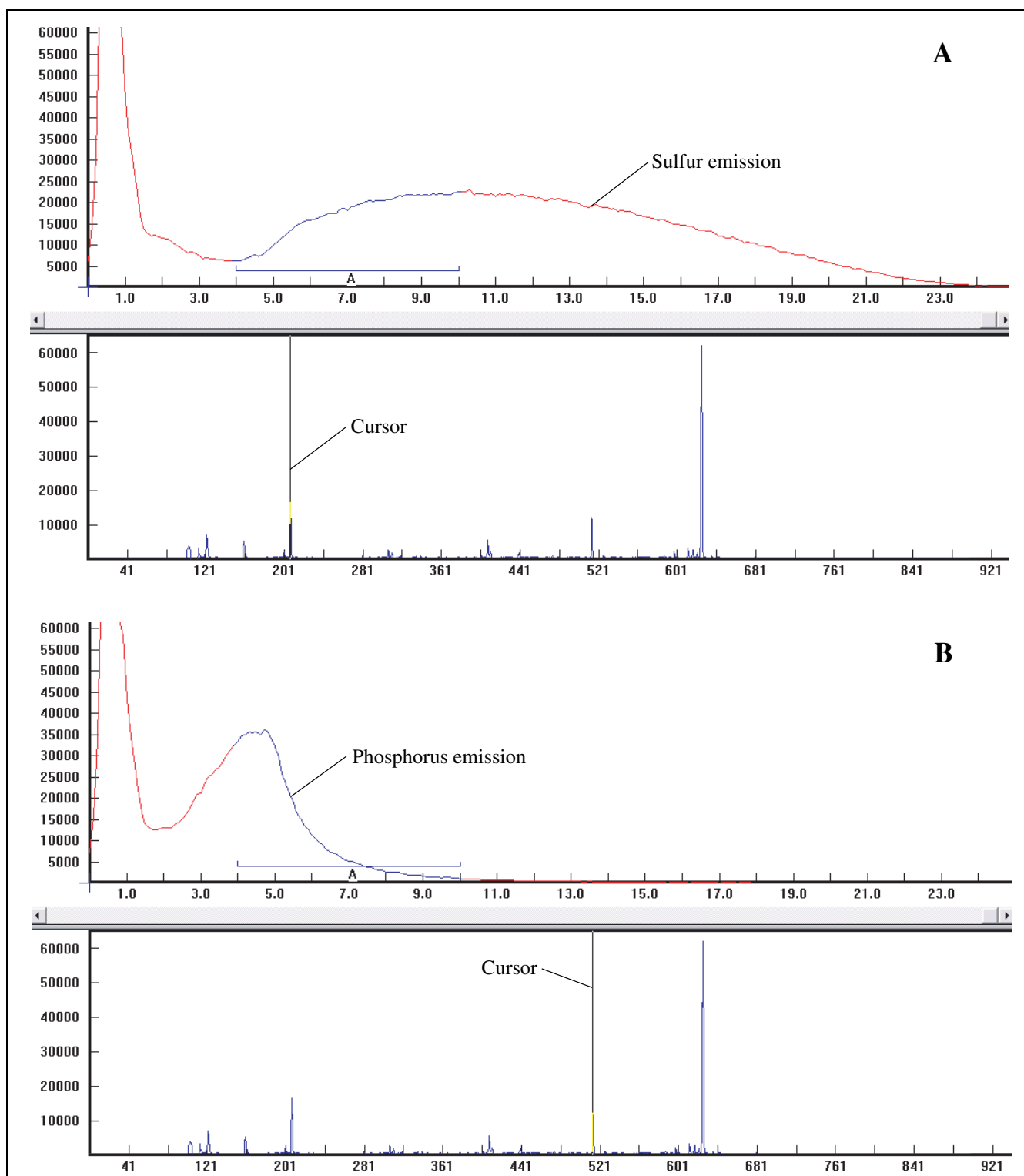


Figure 12. PFPDView screen illustrating the original PFPD phosphorus-mode chromatogram obtained from analysis of a garlic extract spiked with four OP pesticides. The peak at pulse 201 (Figure 12A) is identified as coming from a sulfur compound by examining the corresponding sulfur emission profile in the top half of the screen. The peak at pulse 520 (Figure 12B) is identified as coming from one of the spiked OP pesticides by the distinctly characteristic shape of the corresponding phosphorus emission.

For this example, the interfering sulfur response across the chromatogram was eliminated using the dual gate subtraction technique. Briefly, dual gate subtraction eliminates interference between two species by assigning reciprocal gate assignments (one for phosphorus and one for sulfur), and using a scaling factor, called the Alpha value, to subtract the overlapping portion of the sulfur emission from the phosphorus gate. (For a complete discussion of the dual gate subtraction technique, see OI Analytical Application Note 1127.)

Using PFPDView, reciprocal phosphorus and sulfur gates were defined, and small adjustments were made to the Alpha value until the resulting chromatogram revealed no sulfur interference. Figure 13 shows the reciprocal phosphorus and sulfur gates and Alpha values used in this example. An Alpha value of 1.3 was found to eliminate the observed sulfur interference and result in a “clean” phosphorus chromatogram (Figure 14). This parameter is then entered into the Gate Parameters window in WinPulse and saved to memory for future analyses.

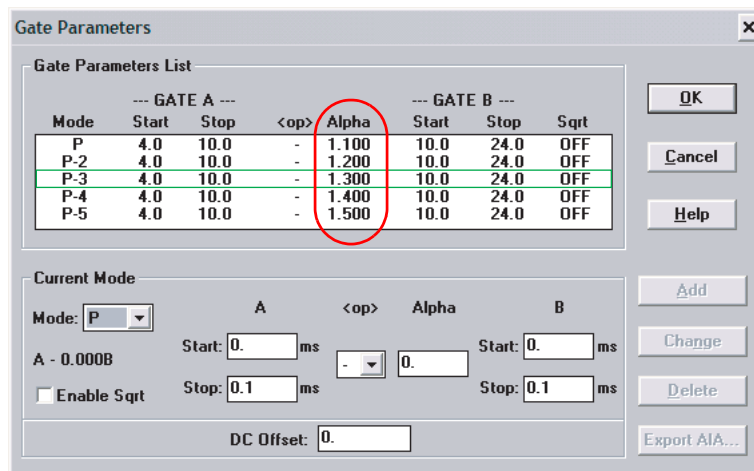


Figure 13. Gate Parameters screen from PFPDView illustrating five different settings for the Alpha value. For this example, the Alpha value of 1.3 produced a phosphorus-only chromatogram with no sulfur interference.

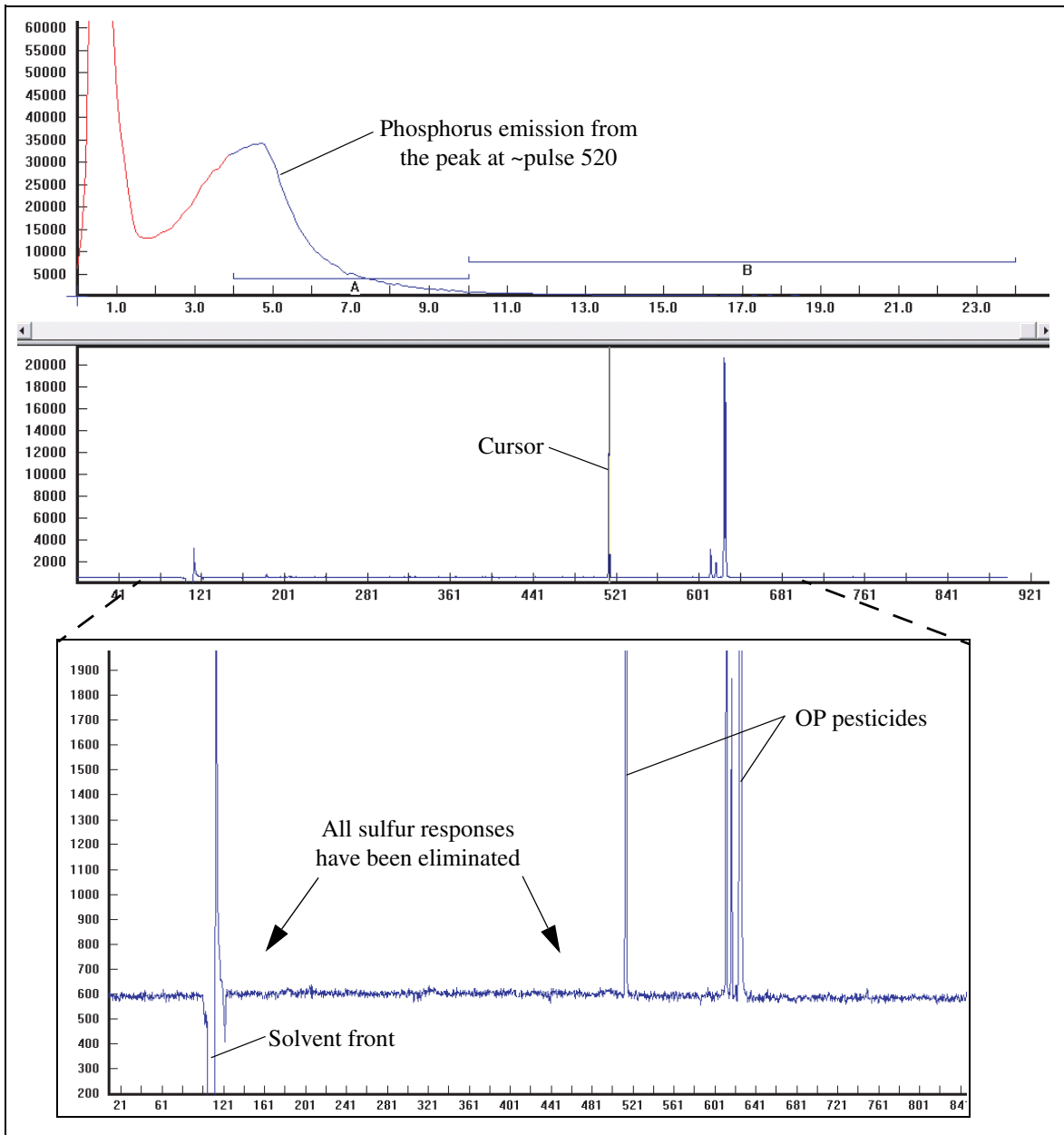


Figure 14. PFPDView screen showing the phosphorus-only chromatogram generated using dual gate subtraction with reciprocal phosphorus and sulfur gates and an Alpha value of 1.3. The insert is an expanded view of the baseline illustrating that all of the sulfur interference has been eliminated.

Phosphorus + Sulfur Dual Element Mode

Since many of the OP pesticides also contain sulfur, the PFPD can be configured with a clear, UV-34 optical filter to collect simultaneous sulfur and phosphorus chromatograms. In this mode, pesticides that contain both sulfur and phosphorus present an emission profile similar to the one shown in Figure 15.

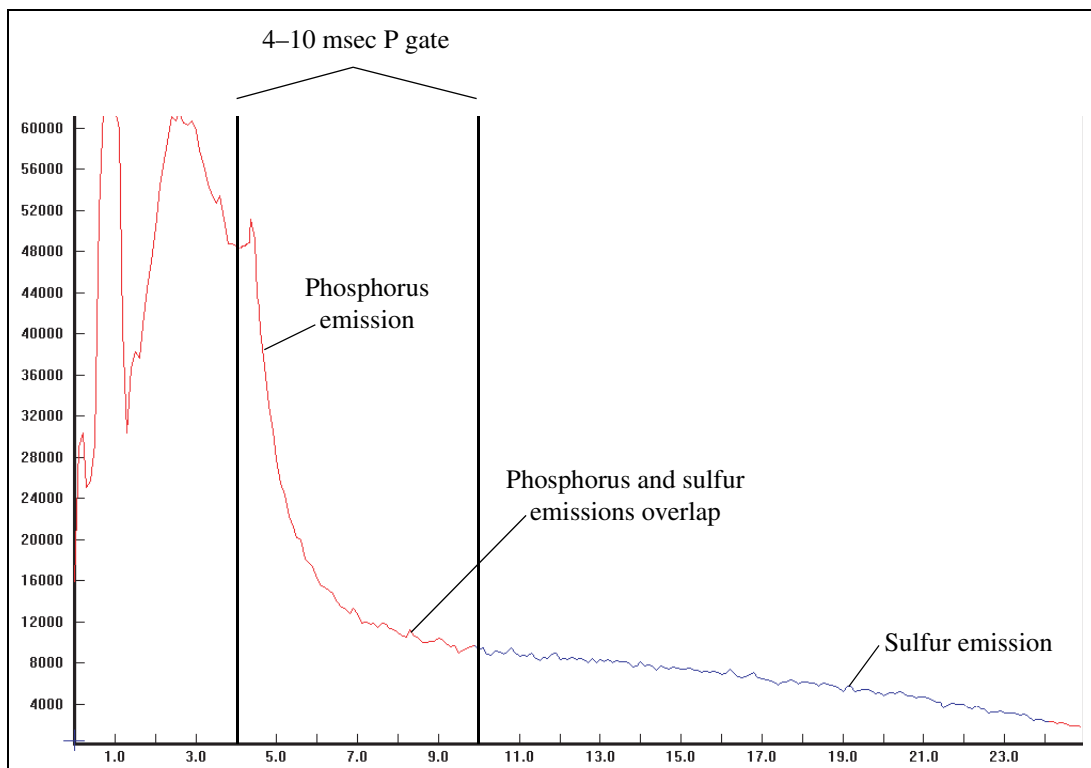


Figure 15. PFPDView screen illustrating the emission profile from a mid-concentration pesticide that contains both phosphorus and sulfur. The PFPD was configured with a clear filter for simultaneous detection of both elements. Note the significant overlap between the sulfur and phosphorus emissions, and the lack of a “pure” phosphorus emission within the 4–10 msec phosphorus gate.

As can be seen in Figure 15, a considerable overlap of the phosphorus and sulfur emissions appears between 6 and 12 msec. A sulfur-only chromatogram can be generated using a gate from 12 to 25 msec, where there is no interfering phosphorus waveform. Configuration of the proper phosphorus gate is slightly more complex, due to the presence of a portion of the sulfur emission within the 4–10 msec phosphorus gate. Dual-gate subtraction must be used to remove this sulfur contribution from the total emission and result in a pure phosphorus chromatogram. Since the phosphorus response is naturally linear and the sulfur response is naturally quadratic, the sulfur emission makes up a disproportionately larger portion of the emission at higher concentrations. But since the overall sulfur emission remains proportional at all concentrations, a single Alpha value can be defined that works throughout the entire phosphorus linear range. The iterative process of defining an Alpha value for simultaneous phosphorus and sulfur detection is exactly as described previously. When used during analysis of standards or samples, this Alpha value produces mutually selective phosphorus and sulfur chromatograms.

In some cases, independent and simultaneous sulfur- and phosphorus-selective chromatograms are desired without employing dual-gate subtraction, such as in chemical warfare agent (CWA) monitoring or when only limited sample is available. The dual-headed PFPD (Figure 16) can be used in these situations. With the dual-headed PFPD, a single PFPD body houses a single combustor and a single combustion process. Two optical filter/PMT combinations are mounted on either side of the body, each configured for selective detection of a single element. Using this configuration, a single GC detector port can be used to collect simultaneous and mutually selective phosphorus and sulfur chromatograms from a single analysis without using dual-gate subtraction.

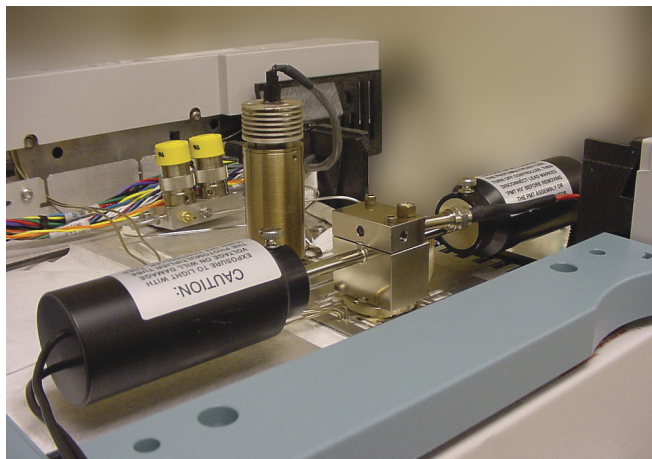


Figure 16. Dual-headed PFPD used for generating simultaneous and mutually selective phosphorus and sulfur chromatograms from a single analysis. This configuration is particularly useful for CWA monitoring or in cases where the sample size may be limited.

Conclusions

The OI Analytical Model 5380 PFPD can be configured to selectively detect and quantify organophosphorus pesticides at single-digit ppb concentrations. The linear calibration range can be extended to 2,000 ppb by using two phosphorus gates, and sulfur interference from complex food matrices can be eliminated using PFPDView software and dual gate subtraction. Simultaneous, mutually selective phosphorus and sulfur chromatograms can be acquired in a single analysis using the dual-headed PFPD.

References

1. OI Analytical Application Note 1127, *Using Dual Gate Subtraction to Enhance the Selectivity of a Pulsed Flame Photometric Detector (PFPD)*.
2. OI Analytical Application Note 1166, *Model 5380 PFPD Photomultiplier Tube and Optical Filter Configurations*.

Dr. Aviv Amirav, Professor of Chemistry at the University of Tel Aviv, Israel, developed and patented the PFPD, and licensed it to O.I. Corporation.

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