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Method 622.1: The Determination of Thiophosphate Pesticides in Municipal and Industrial Wastewater

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1. *SCOPE AND APPLICATION*

1.1 This method covers the determination of certain thiophosphate pesticides. The following parameters can be determined by this method:

Parameter	***CAS No.***
Aspon	3244-90-4
Dichlofenthion	97-17-6
Famphur	52-85-7
Fenitrothion	122-14-5
Fonophos	944-22-9
Phosmet	732-11-6
Thionazin	297-97-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 2. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in certain other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph / mass spectrometer (GC / MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. *SUMMARY OF METHOD*

2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector gas chromatography (GC/AFD).[1]

2.2 This method provides a Florisil column cleanup procedure to aid in the elimination of interferences that may be encountered.

3. *INTERFERENCES*

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap and reagent water. Drain dry and heat in an oven or muffle furnace at 400°C for 15-30 minutes. Do not heat volumetric glassware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDLs listed in Table 2.

4. *SAFETY*

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[3-5] for the information of the analyst.

5. *Apparatus and Materials*

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1 qt or 1 L volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsing with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000 mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250 mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials: Amber glass, 10-15 mL capacity with PTFE-lined screw-cap.

5.2.9 Erlenmeyer flask: 250 mL.

5.2.10 Graduated cylinder: 1000 mL.

5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat at 400°C for four hours or extract in a Soxhlet with methylene chloride.

5.4 Water bath: Heated, capable of temperature control (±2°C). The bath should be used in a hood.

5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector: Alkali flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic-specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, methanol, petroleum ether, anhydrous ethyl ether, and acetone: Distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant Test Strips (available from Scientific Products Co., Catalog No. P 1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.4 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in a brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160-170°C. Seal tightly with PTFE or aluminum-foil-lined screw-cap and cool to room temperature.

6.5 6N sodium hydroxide.

6.6 6N sulfuric acid.

6.7 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality ethyl ether and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with ethyl ether. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1-5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with ethyl ether. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.3.2 Using injections of 1-5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)\ (C_{is})}{(A_{is})\ (C_s)}$$

where

A_s = *Response for the parameter to be measured*
A_{is} = *Response for the internal standard*
C_{is} = *Concentration of the internal standard, in µg/L*
C_s = *Concentration of the parameter to be measured, in µg/L*

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s / A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000 mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R) and the standard deviation of the percent recovery (s) for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 3, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = $R + 3s$
Lower Control Limit (LCL) = $R - 3s$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.[1]

8.5 Before processing any samples, the analyst should demonstrate though the analysis of a 1 L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. *Sample Collection, Preservation, and Handling*

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 Adjust the pH of the sample to 6-8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. *Sample Extraction*

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6-8 with 6N sodium hydroxide or 6N sulfuric acid.

10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask.

10.3 Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20-30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30-40 mL of methylene chloride.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60-65°C, so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of methylene chloride. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.

10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than three days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with the gas chromatographic analysis in Section 12. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that reported in Table 3.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the seven thiophosphate pesticides listed in Table 1.

11.2.1 Add 20 g of Florisil to 100 mL of ethyl ether and 400 µL of reagent water in a 250 mL Erlenmeyer flask. Shake vigorously for 15 minutes. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Wash the column with 25 mL of petroleum ether. Use a column flow of 2-2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.

11.2.2 Quantitatively add the sample extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 6% ethyl ether in petroleum ether. Discard this fraction.

11.2.3 Elute the column with 50 mL of 15% ethyl ether in petroleum ether (Fraction 1) and collect eluate in a K-D apparatus. Repeat process with 50 mL of 50% ethyl ether in petroleum ether (Fraction 2), 50 mL of 100% ethyl ether (Fraction 3), 50 mL 6% acetone in ethyl ether (Fraction 4), and 100 mL 15% acetone in ethyl ether (Fraction 5), collecting each fraction in a separate K-D apparatus. The elution patterns for the thiophosphates are shown in Table 1. Concentrate each fraction to 1 mL as described in Sections 10.6 and 10.7. Proceed with gas chromatographic analysis.

11.2.4 The above-mentioned fractions can be combined before concentration at the discretion of the analyst.

12. GAS CHROMATOGRAPHY

12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the gas chromatographic system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1-5 µL of the sample extract using the solvent flush technique.[8] Record the volume injected to the nearest 0.05 µL, and record the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention-time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$Concentration,\ \mu g/L = \frac{(A)\ (V_t)}{(V_i)\ (V_s)}$$

where
A = *Amount of material injected, in ng*
V_i = *Volume of extract injected, in µL*
V_t = *Volume of total extract, in µL*
V_s = *Volume of water extracted, in mL*

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$Concentration,\ \mu g/L = \frac{(A_s)\ (I_s)}{(A_{is})\ (RF)\ (V_o)}$$

where
A_s = *Response for parameter to be measured*
A_{is} = *Response for the interal standard*
I_s = *Amount of internal standard added to each extract, in µg*
V_o = *Volume of water extracted, in L*

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least five scans per peak but not to exceed seven seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC-to-MS interface constructed of all glass or glass-lined materials is recommended. When using a fused-silica capillary column, the column outlet should be threaded through the interface to within a few millimeters of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[10]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.[9]

14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to ±10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[11] The MDL concentrations listed in Table 2 were obtained using reagent water.[1] Similar results were achieved using representative wastewaters.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 x MDL to 1000 x MDL.

15.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after Florisil cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.[1]

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Table 1. Elution Orders and Recoveries of Thiophosphates from Florisil

	Recovery in Specified Fraction (%)[a]							
Compound	***F1***	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***Total***
Aspon			94	2				96
Dichlofenthion			92					92
Famphur						6	103	109
Fenitrothion				51	55			106
Fonophos			84	6				90
Phosmet						37	69	106
Thionazine				2	93			95

[a]Results of single determination with 100 µg of each compound. Elution solvents were 50 mL each of the following:

F1 = 2% methylene chloride in petroleum ether
F2 = 6% ethyl ether in petroleum ether
F3 = 15% ethyl ether in petroleum ether
F4 = 50% ethyl ether in petroleum ether
F5 = 100% ethyl ether
F6 = 6% acetone in ethyl ether
F7 = 15% acetone in ethyl ether

Table 2. Chromatographic Conditions and Estimated Method Detection Limits

Parameter	***Retention Time (min)***		***MDL (µg/L)***
	Column 1	***Column 2***	
Thionazin	18.3	25.0	1
Fonophos	20.5	27.8	0.7
Dichlofenthion	21.4	29.4	0.7
Aspon	22.6	30.2	0.6
Fenitrothion	23.1	30.8	2
Famphur	28.1	34.8	19
Phosmet	30.0	36.2	1

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a glass column 1.8 m long by 2 mm ID with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80-300°C at 8°C/min with a four minute hold at each extreme, injector temperature is 250°C and detector is 300°C. Alkali flame detector at bead voltage of 16 volts.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2100 packed in a glass column 1.8 m long by 2 mm ID with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80-300°C at 8°C/min with a four minute hold at each extreme, injector temperature is 250°C and detector is 300°C.

Table 3. Single-Laboratory Accuracy and Precision[a]

Parameter	***Sample Type[b]***	***Background (µg/L)[c]***	***Spike (µg/L)***	***Mean Recovery (%)***	***Relative Standard Deviation (%)***	***Number of Replicates***
Aspon	1	ND	50	83	7	7
	2	ND	500	87	3	7
Dichlofenthion	1	ND	50	83	7	7
	2	ND	500	84	4	7
Famphur	1	ND	50	86	6	7
	2	ND	500	86	4	7
Fenitrothion	1	ND	50	82	7	7
	2	ND	500	83	4	7
Fonophos	1	ND	50	84	7	7
	2	ND	500	86	4	7
Phosmet	1	ND	50	85	5	7
	2	ND	500	87	5	7
Thionizin	1	ND	50	84	7	7
	2	ND	500	89	5	7

[a]Column 1 conditions were used.
[b]1 = Low-level relevant industrial effluent
2 = Municipal sewage influent
[c]ND = Not detected

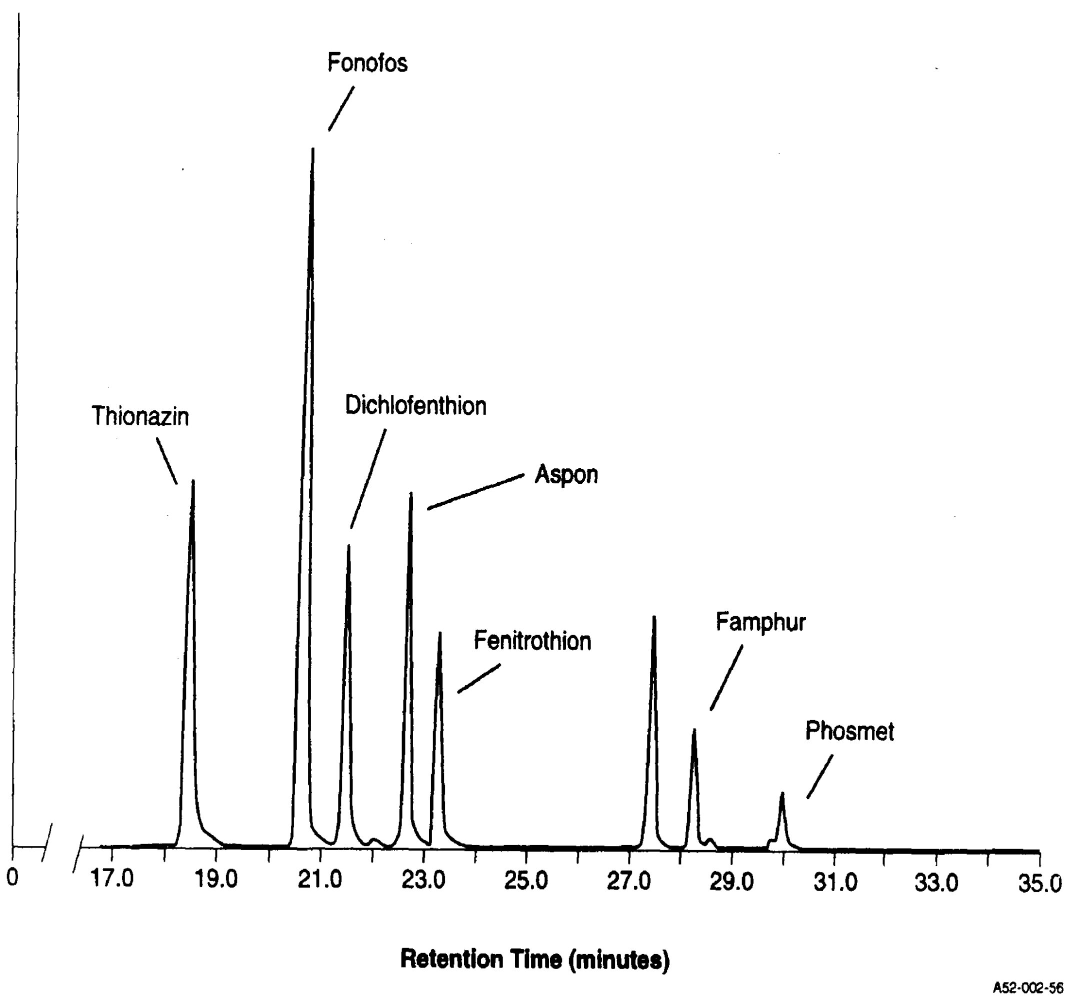

Figure 1. GC-AFD Chromatogram of 100 ng Each of Seven Thiophosphates (Column 1)

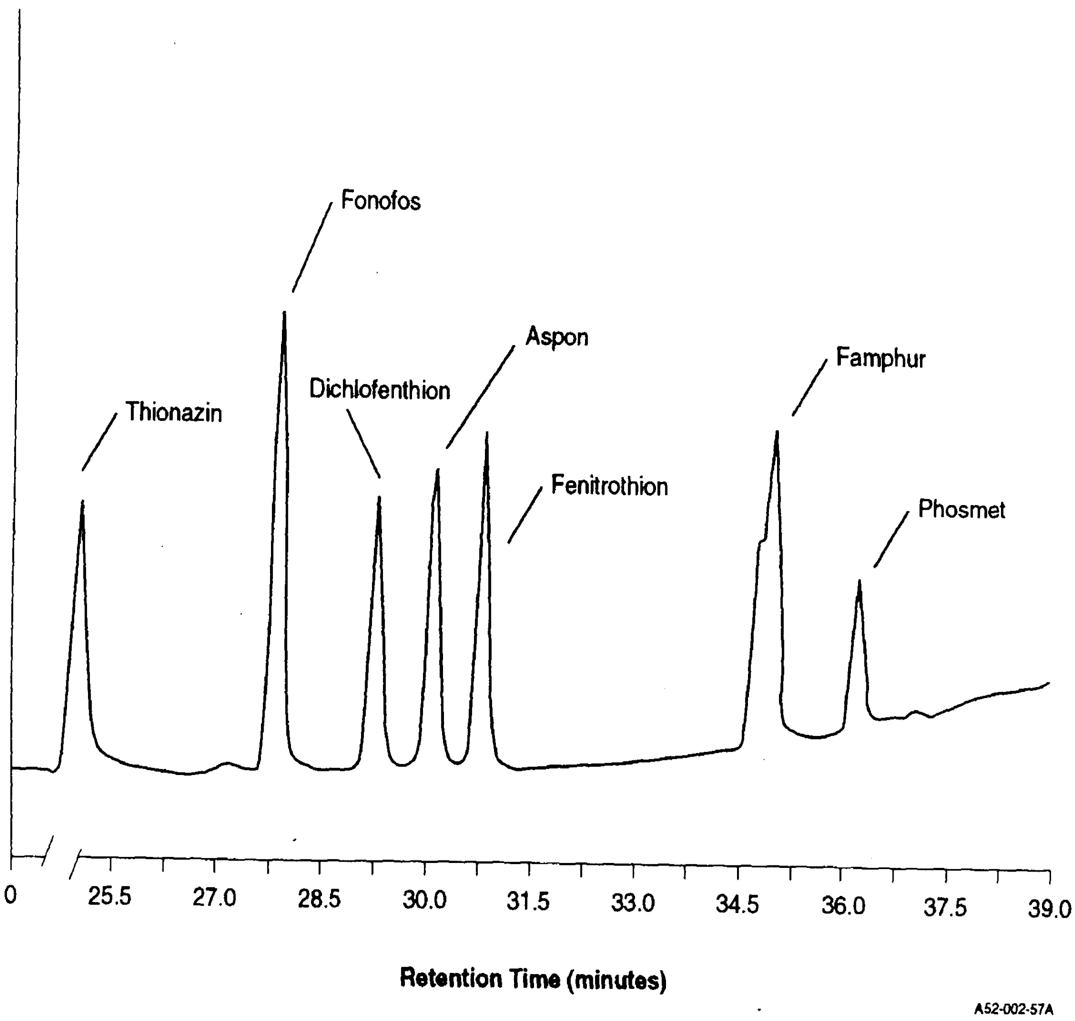

Figure 2. GC-FID Chromatogram of 100 ng Each of Seven Thiophosphates (Column 2)