METHOD 8140

ORGANOPHOSPHORUS PESTICIDES

1.0 SCOPE AND APPLICATION

1.1 Method 8140 is a gas chromatographic (GC) method used to determine the concentration of various organosphosphorus pesticides. Table 1 indicates compounds that may be determined by this method and lists the method detection limit for each compound in reagent water. Table 2 lists the practical quantitation limit (PQL) for other matrices.

1.2 When Method 8140 is used to analyze unfamiliar samples, compound identifications should be supported by at least two additional qualitative techniques if mass spectroscopy is not employed. Section 8.4 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2.0 SUMMARY OF METHOD

2.1 Method 8140 provides gas chromatographic conditions for the detection of ppb levels of organophosphorus pesticides. Prior to analysis, appropriate sample extraction techniques must be used. Both neat and diluted organic liquids (Method 3580, Waste Dilution) may be analyzed by direct injection. A 2- to 5-uL aliquot of the extract is injected into a gas chromatograph, and compounds in the GC effluent are detected with a flame photometric or thermionic detector.

2.2 If interferences are encountered in the analysis, Method 8140 may also be performed on extracts that have undergone cleanup using Method 3620 and/or Method 3660.

3.0 INTERFERENCES

3.1 Refer to Methods 3500 (Section 3.5, in particular), 3600, and 8000.

3.2 The use of Florisil cleanup materials (Method 3620) for some of the compounds in this method has been demonstrated to yield recoveries less than 85% and is therefore not recommended for all compounds. Refer to Table 2 of Method 3620 for recoveries of organophosphorous pesticides as a function of Florisil fractions. Use of phosphorus- or halogen-specific detectors, however, often obviates the necessity for cleanup for relatively clean sample matrices. 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 analyte is no less than 85%.

TABLE 1. GAS CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS FOR ORGANOPHOSPHOROUS PESTICIDES[a]

Compound	GC column[b]	Retention time (min)	Method detection limit (ug/L)
Azinphos-methyl	1a	6.80	1.5
Bolstar	1a	4.23	0.15
Chlorpyrifos	2	6.16	0.3
Coumaphos	1a	11.6	1.5
Demeton-O	1a	2.53	0.25
Demeton-S	1a	1.16	0.25
Diazinon	2	7.73	0.6
Dichlorvos	1b, 3	0.8, 1.50	0.1
Disulfoton	1a	2.10	0.20
Ethoprop	2	3.02	0.25
Fensulfothion	1a	6.41	1.5
Fenthion	1a	3.12	0.10
Merphos	2	7.45	0.25
Mevinphos	1b	2.41	0.3
Naled	3	3.28	0.1
Parathion methyl	2	3.37	0.03
Phorate	1a	1.43	0.15
Ronnel	2	5.57	0.3
Stirophos (Tetrachlorvinphos)	1b, 3	8.52, 5.51	5.0
Tokuthion (Prothiofos)	1a	3.40	0.5
Trichloronate	1a	2.94	0.15

[a]Development of Analytical Test Procedures for Organic Pollutants in Wastewater; Report for EPA Contract 68-03-2711 (in preparation).

[b]See Sections 4.2.1 and 7.2 for column descriptions and conditions.

TABLE 2. DETERMINATION OF PRACTICAL QUANTITATION LIMITS (PQL) FOR VARIOUS MATRICES[a]

Matrix	Factor[b]
Ground water	10
Low-level soil by sonication with GPC cleanup	670
High-level soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[a]Sample PQLs are highly matrix-dependent. The PQLs listed herein are provided for guidance and may not always be achievable.

[b]PQL = [Method detection limit (Table 1)] X [Factor (Table 2)]. For non-aqueous samples, the factor is on a wet-weight basis.

3.3 Use of a flame photometric detector in the phosphorus mode will minimize interferences from materials that do not contain phosphorus. Elemental sulfur, however, may interfere with the determination of certain organophosphorus pesticides by flame photometric gas chromatography. Sulfur cleanup using Method 3660 may alleviate this interference.

3.4 A halogen-specific detector (i.e., electrolytic conductivity or microcoulometric) is very selective for the halogen-containing pesticides and is recommended for use with dichlorvos, naled, and stirophos.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injections and all required accessories, including detectors, column supplies, recorder, gases, and syringes. A data system for measuring peak areas and/or peak heights is recommended.

4.1.1 **Columns:**

4.1.1.1 Column 1a and 1b: 1.8-m x 2-mm I.D. glass, packed with 5% SP-2401 on Supelcoport, 100/120 mesh (or equivalent).

4.1.1.2 Column 2: 1.8-m x 2-mm I.D. glass, packed with 3% SP-2401 on Supelcoport, 100/120 mesh (or equivalent).

4.1.1.3 Column 3: 50-cm x 1/8-in O.D. Teflon, packed with 15% SE-54 on Gas Chrom Q, 100/120 mesh (or equivalent).

4.1.2 **Detectors:** The following detectors have proven effective in analysis for the analytes listed in Table 1 and were used to develop the accuracy and precision statements in Section 9.0.

4.1.2.1 Phosphorus-specific: Nitrogen/Phosphorus (N/P), operated in phosphorus-sensitive mode.

4.1.2.2 Flame Photometric (FPD): FPD is more selective for phosphorus than the N/P.

4.1.2.3 Halogen-specific: Electrolytic conductivity or microcoulometric. These are very selective for those pesticides containing halogen substituents.

4.2 Balance: analytical, capable of accurately weighing to the nearest 0.0001 g.

4.3 Vials: Amber glass, 10- to 15-mL capacity with Teflon-lined screw-cap.

4.4 Kuderna-Danish (K-D) apparatus:

4.4.1 **Concentrator tube:** 10-mL, graduated (Kontes K-570050-1025 or equivalent). Ground-glass stopper is used to prevent evaporation of extracts

4.4.2 **Evaporation flask:** 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs.

4.4.3 **Snyder column:** Three-ball macro (Kontes K-503000-0121 or equivalent).

4.4.4 **Snyder column:** Two-ball micro (Kontes K-569001-0219 or equivalent).

4.5 Boiling chips: Solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent).

4.6 Water bath: Heated, with concentric ring cover, capable of temperature control (±5°C). The bath should be used in a hood.

4.7 Microsyringe: 10-uL.

4.8 Syringe: 5-mL.

4.9 Volumetric flasks: 10-, 50-, and 100-mL, ground-glass stopper.

5.0 REAGENTS

5.1 Solvents: Hexane, acetone, isooctane (2,2,4-trimethylpentane) (pesticide quality or equivalent).

5.2 Stock standard solutions:

5.2.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in hexane or other suitable solvent 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.

5.2.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.2.3 Stock standard solutions must be replaced after one year, or sooner if comparison with check standards indicates a problem.

5.3 Calibration standards: Calibration standards at a minimum of five concentration levels for each parameter of interest should be prepared through dilution of the stock standards with isooctane. One of the concentration levels should be at a concentration near, but above, the method detection limit. The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Calibration standards must be replaced after six months, or sooner if comparison with check standards indicates a problem.

5.4 Internal standards (if internal standard calibration is used): To use this approach, the analyst must select one or more internal standards that are 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. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

5.4.1 Prepare calibration standards at a minimum of five concentration levels for each parameter of interest as described in Paragraph 5.3.

5.4.2 To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable solvent.

5.4.3 Analyze each calibration standard according to Section 7.0.

5.5 Surrogate standards: The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and reagent water blank with one or two surrogates (e.g., organophosphorous pesticides not expected to be present in the sample) recommended to encompass the range of the temperature program used in this method. Deuterated analogs of analytes should not be used as surrogates for gas chromatographic analysis due to coelution problems.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Section 4.1. Extracts must be stored under refrigeration and analyzed within 40 days of extraction.

7.0 PROCEDURE

7.1 Extraction:

7.1.1 Refer to Chapter Two for guidance on choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral, or as is, pH with methylene chloride, using either Method 3510 or 3520. Solid samples are extracted using either Method 3540 or 3550.

7.1.2 Prior to gas chromatographic analysis, the extraction solvent may be exchanged to hexane. This is recommended if the detector used is halogen-specific. The exchange is performed during the K-D procedures listed in all of the extraction methods. The exchange is performed as follows.

7.1.2.1 Following K-D of the methylene chloride extract to 1 mL using the macro-Snyder column, allow the apparatus to cool and drain for at least 10 min.

7.1.2.2 Momentarily remove the Snyder column, add 50 mL of hexane, a new boiling chip, and reattach the macro-Snyder column. Concentrate the extract using 1 mL of hexane to prewet the Snyder column. Place the K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete concentration in 5-10 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. 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 min.

7.1.2.3 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of hexane. A 5-mL syringe is recommended for this operation. Adjust the extract volume to 10.0 mL. Stopper the concentrator tube and store refrigerated at 4°C if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial. Proceed with gas chromatographic analysis if further cleanup is not required.

7.2 Gas chromatography conditions (Recommended):

7.2.1 **Column 1a:** Set helium carrier gas flow at 30 mL/min flow rate. Column temperature is set at 150°C for 1 min and then programmed at 25°C/min to 220°C and held.

7.2.2 **Column 1b:** Set nitrogen carrier gas flow at 30 mL/min flow rate. Column temperature is set at 170°C for 2 min and then programmed at 20°C/min to 220°C and held.

7.2.3 **Column 2:** Set helium carrier gas at 25 mL/min flow rate. Column temperature is set at 170°C for 7 min and then programmed at 10°C/min to 250°C and held.

7.2.4 **Column 3:** Set nitrogen carrier gas at 30 mL/min flow rate. Column temperature is set at 100°C and then immediately programmed at 25°C/min to 200°C and held.

7.3 Calibration: Refer to Method 8000 for proper calibration techniques. Use Table 1 and especially Table 2 for guidance on selecting the lowest point on the calibration curve.

7.3.1 The procedure for internal or external calibration may be used. Refer to Method 8000 for a description of each of these procedures.

7.3.2 If cleanup is performed on the samples, the analyst should process a series of standards through the cleanup procedure and then analyze the samples by GC. This will confirm elution patterns and the absence of interferents from the reagents.

7.4 Gas chromatographic analysis:

7.4.1 Refer to Method 8000. If the internal standard calibration technique is used, add 10 uL of internal standard to the sample prior to injection.

7.4.2 Follow Section 7.6 in Method 8000 for instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 10 samples in the analysis sequence.

7.4.3 Examples of chromatograms for various organophosphorous pesticides are shown in Figures 1 through 4.

7.4.4 Record the sample volume injected and the resulting peak sizes (in area units or peak heights).

7.4.5 Using either the internal or external calibration procedure (Method 8000), determine the identity and quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes. See Section 7.8 of Method 8000 for calculation equations.

7.4.6 If peak detection and identification are prevented due to interferences, the hexane extract may undergo cleanup using Method 3620. The resultant extract(s) may be analyzed by GC directly or may undergo further cleanup to remove sulfur using Method 3660.

7.5 Cleanup:

7.5.1 Proceed with Method 3620, followed by, if necessary, Method 3660, using the 10-mL hexane extracts obtained from Paragraph 7.1.2.3.

7.5.2 Following cleanup, the extracts should be analyzed by GC, as described in the previous paragraphs and in Method 8000.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. Quality control to validate sample extraction is covered in Method 3500 and in the extraction method utilized. If extract cleanup was performed, follow the QC in Method 3600 and in the specific cleanup method.

8.2 Procedures to check the GC system operation are found in Method 8000, Section 8.6.

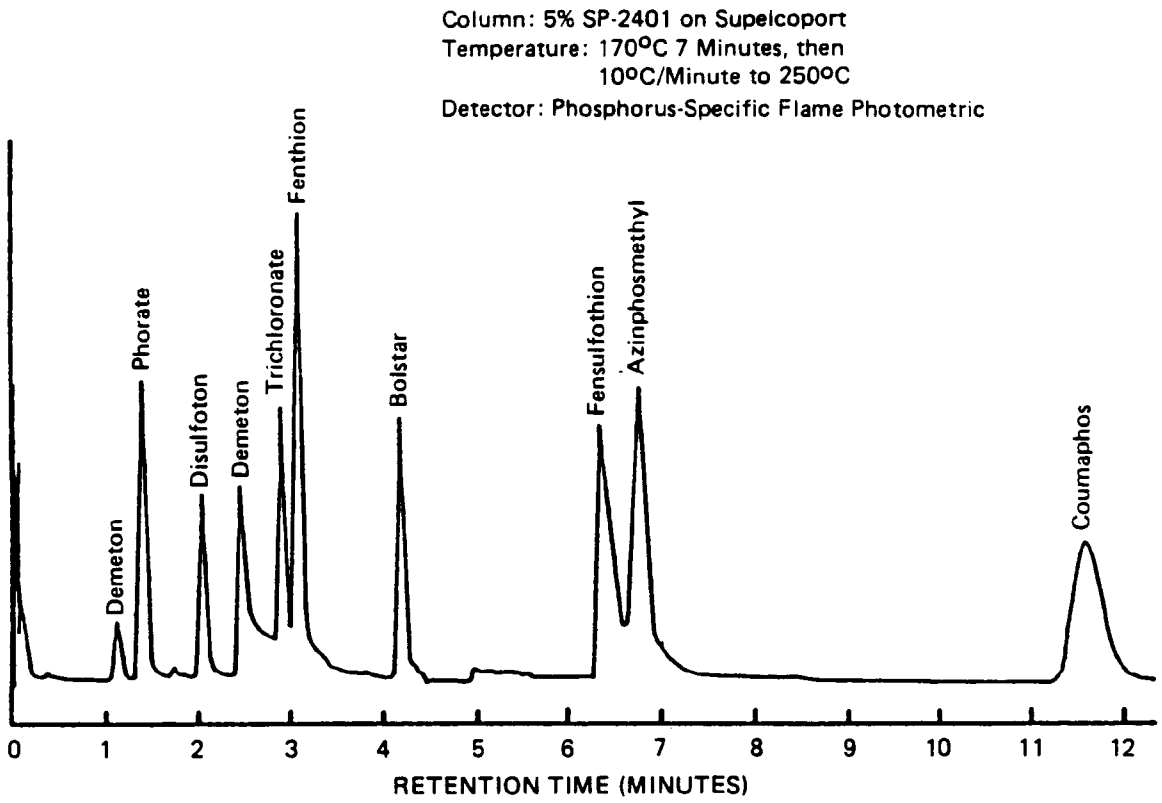


Figure 1. Gas chromatogram of organophosphorus pesticides (Example 1).

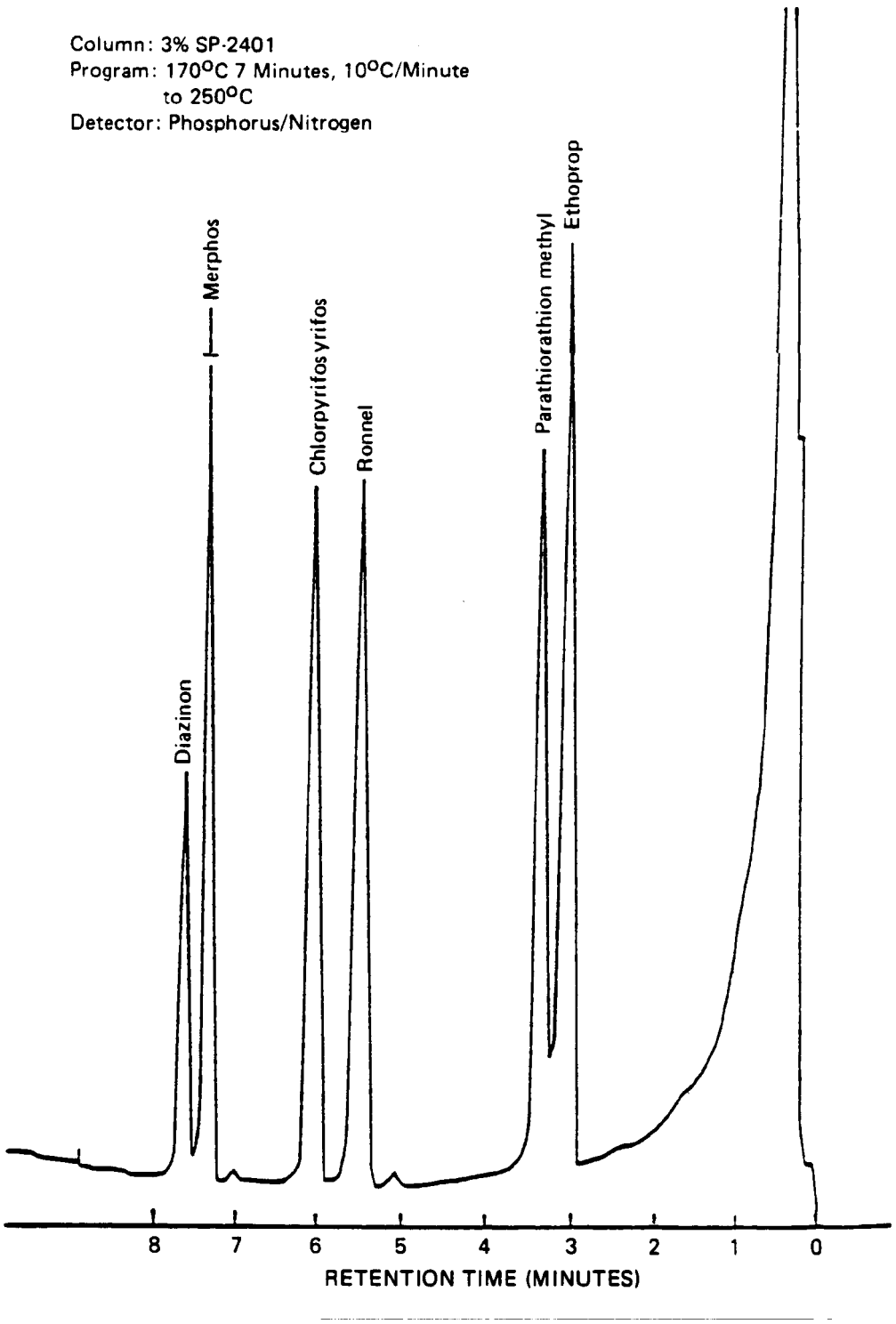


Figure 2. Gas chromatogram of organophosphorus pesticides (Example 2).

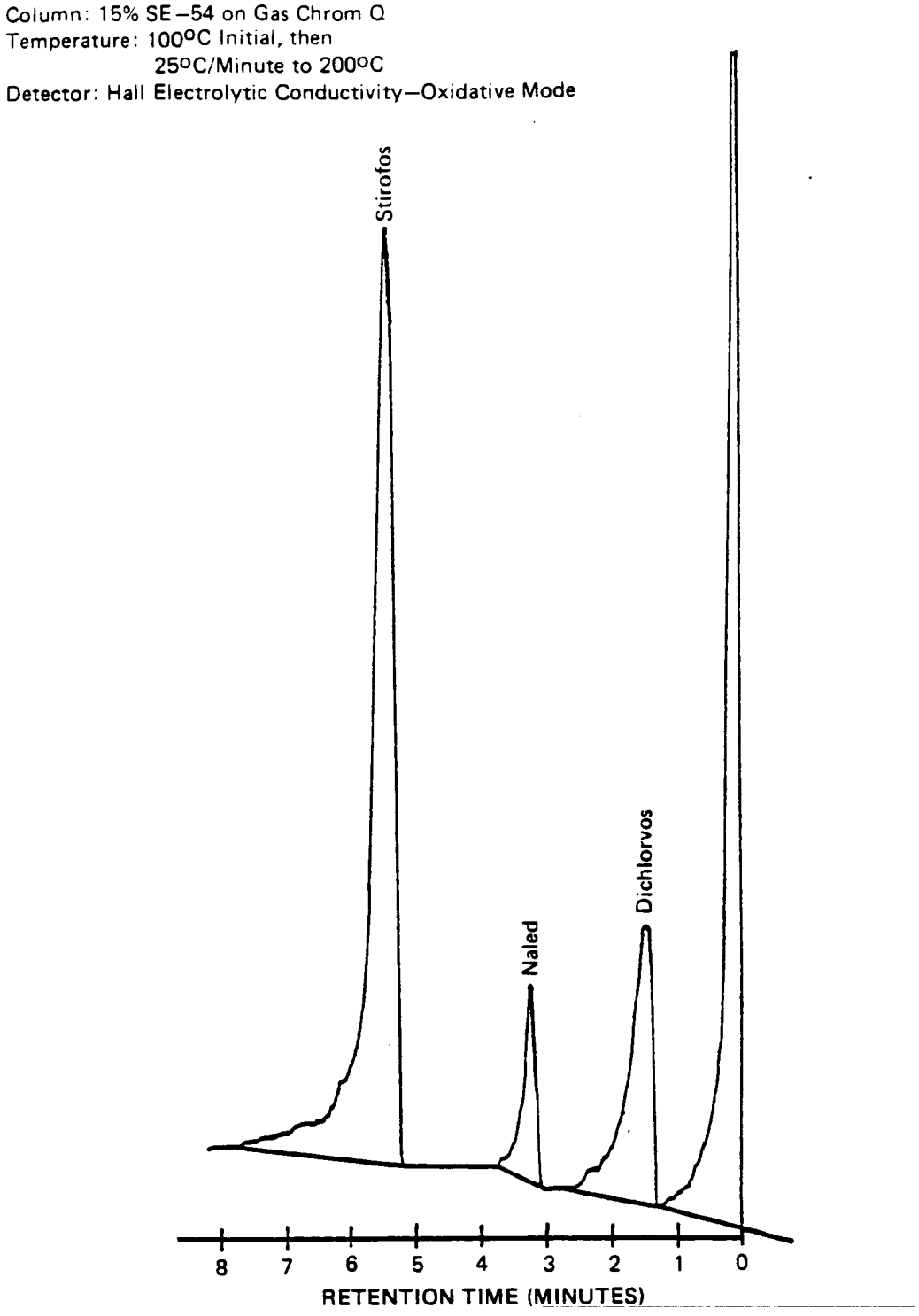


Figure 3. Gas chromatogram of organophosphorus pesticides (Example 3).

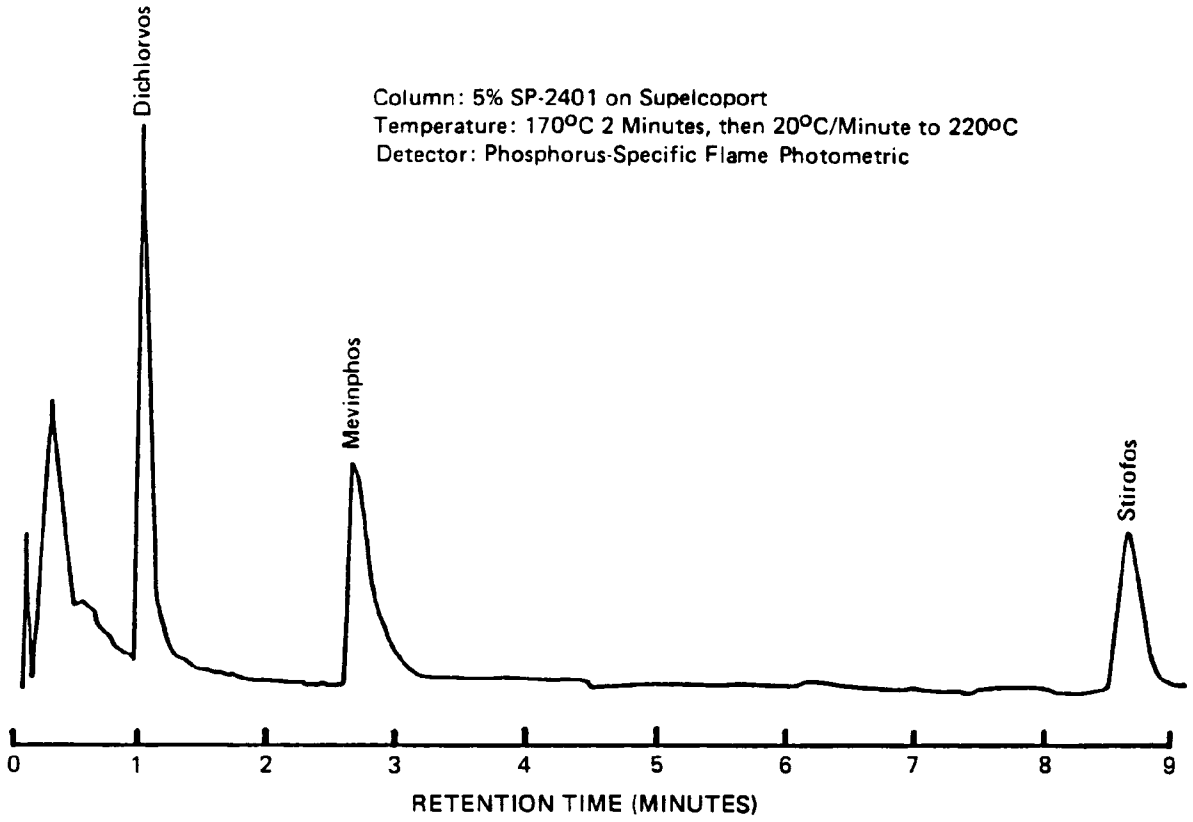


Figure 4. Gas chromatogram of organophosphorus pesticides (Example 4).

8.2.1 Select a representative spike concentration for each analyte to be measured. The quality control check sample concentrate (Method 8000, Section 8.6) should contain each analyte in acetone at a concentration 1,000 times more concentrated than the selected spike concentration.

8.2.2 Table 3 indicates Single Operator Accuracy and Precision for this method. Compare the results obtained with the results given in Table 3 to determine if the data quality is acceptable.

8.3 Calculate surrogate standard recovery on all samples, blanks, and spikes. Determine if the recovery is within limits (limits established by performing QC procedures outlined in Method 8000, Section 8.10).

8.3.1 If recovery is not within limits, the following procedures are required.

- Check to be sure there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.

- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.

- Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration."

8.4 GC/MS confirmation:

8.4.1 GC/MS techniques should be judiciously employed to support qualitative identifications made with this method. The GC/MS operating conditions and procedures for analysis are those specified in Method 8270.

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

8.4.3 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These steps may include the use of alternate packed or capillary GC columns and additional cleanup.

9.0 METHOD PERFORMANCE

9.1 Single-operator accuracy and precision studies have been conducted using spiked wastewater samples. The results of these studies are presented in Table 3.

10.0 REFERENCES

1. Pressley, T.A. and J.E. Longbottom, "The Determination of Organophosphorus Pesticides in Industrial and Municipal Wastewater: Method 614," U.S. EPA/EMSL, Cincinnati, OH, EPA-600/4-82-004, 1982.

2. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," Journal of the Association of Official Analytical Chemists 48, 1037, 1965.

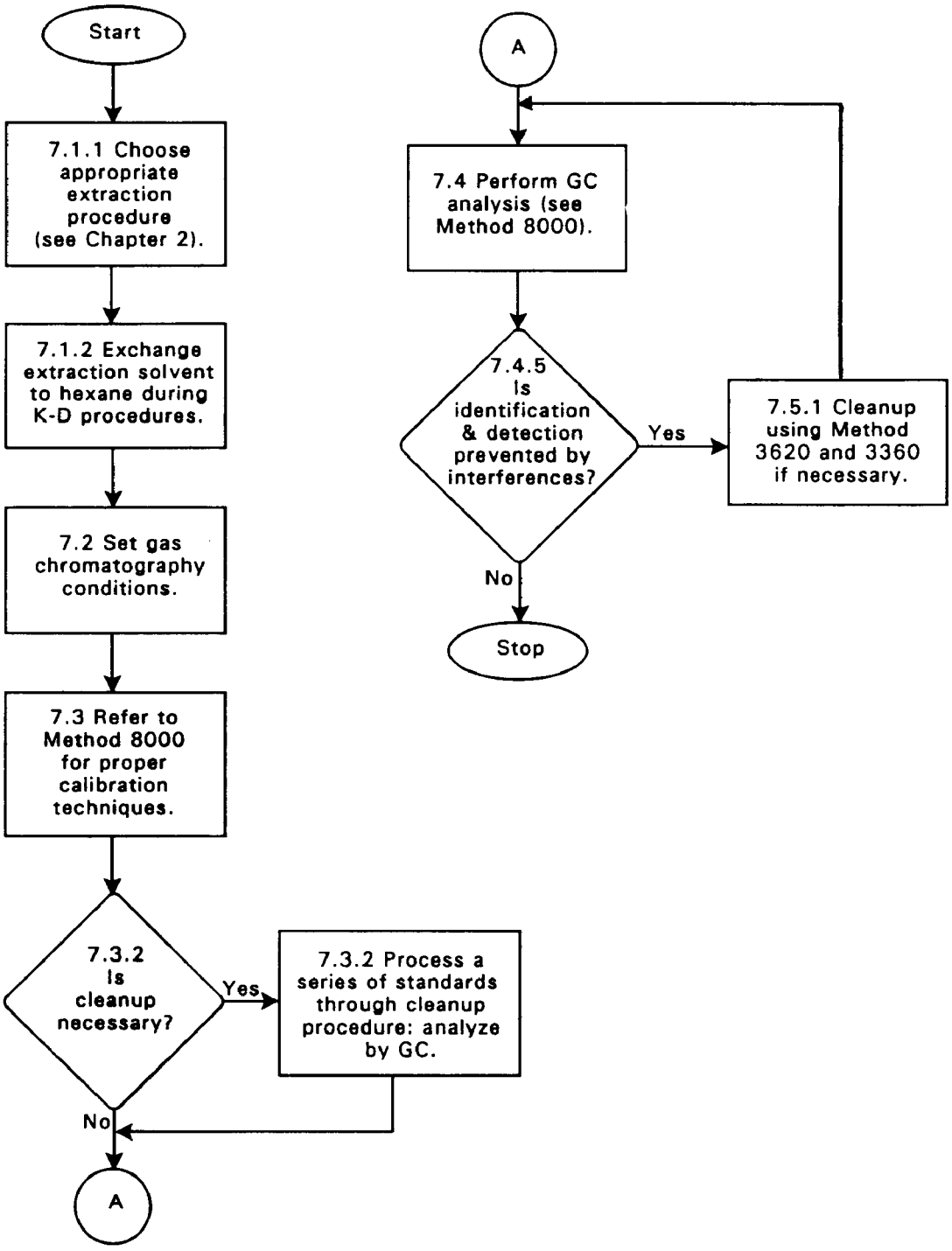
3. U.S. EPA, "Analysis of Volatile Hazardous Substances by GC/MS: Pesticide Methods Evaluation," Letter Reports 6, 12A, and 14, EPA Contract 68-03-2697, 1982.

4. U.S. EPA, "Method 622, Organophosphorous Pesticides," Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

TABLE 3. SINGLE-OPERATOR ACCURACY AND PRECISION[a]

Parameter	Average recovery (%)	Standard deviation (%)	Spike range (ug/L)	Number of analyses
Azinphos-methyl	72.7	18.8	21-250	17
Bolstar	64.6	6.3	4.9-46	17
Chlorpyrifos	98.3	5.5	1.0-50.5	18
Coumaphos	109.0	12.7	25-225	17
Demeton	67.4	10.5	11.9-314	17
Diazinon	67.0	6.0	5.6	7
Dichlorvos	72.1	7.7	15.6-517	16
Disulfoton	81.9	9.0	5.2-92	17
Ethoprop	100.5	4.1	1.0-51.5	18
Fensulfothion	94.1	17.1	23.9-110	17
Fenthion	68.7	19.9	5.3-64	17
Merphos	120.7	7.9	1.0-50	18
Mevinphos	56.5	7.8	15.5-520	16
Naled	78.0	8.1	25.8-294	16
Parathion methyl	96.0	5.3	0.5-500	21
Phorate	62.7	8.9	4.9-47	17
Ronnel	99.2	5.6	1.0-50	18
Stirophos	66.1	5.9	30.3-505	16
Tokuthion	64.6	6.8	5.3-64	17
Trichloronate	105.0	18.6	20	3

[a]Information taken from Reference 4.


Start
7.1.1 Choose appropriate extraction procedure (see Chapter 2).
7.1.2 Exchange extraction solvent to hexane during K-D procedures.
7.2 Set gas chromatography conditions.
7.3 Refer to Method 8000 for proper calibration techniques.
7.3.2 Is cleanup necessary?
Yes
7.3.2 Process a series of standards through cleanup procedure: analyze by GC.
No
A
A
7.4 Perform GC analysis (see Method 8000).
7.4.5 Is identification & detection prevented by interferences?
Yes
7.5.1 Cleanup using Method 3620 and 3360 if necessary.
No
Stop