



Residue Method M-1753.01
Author D. Kim
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Supercedes None
Effective Date 8/25/87
Approved J. Boyd
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AMERICAN CYANAMID COMPANY
AGRICULTURAL RESEARCH DIVISION
CHEMICAL DEVELOPMENT
P.O. Box 400
Princeton, NJ 08540

Recommended Method of Analysis

CYTHON® malathion (CL 6,601): GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Corn Forage, Silage, Fodder, and Grain

A. Principle

Residues of malathion (CL 6,601) and malaoxon (CL 28,967) are extracted from finely ground plant tissue with acetonitrile. The filtered extracts are subjected to cleanup procedures involving treatment with activated charcoal and passage of a methylene chloride-acetone solution through a disposable silica-gel solid phase extraction cartridge. The malathion (CL 6,601) and malaoxon (CL 28,967) concentrations are determined by gas chromatography using an instrument equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated by direct comparison of peak height to those of external standards. The validated sensitivity of the method is 0.05 ppm for each compound.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
2. Waring Blendor: Model 31BL46 with 1-quart capacity glass blender jar (Waring Products Division, Dynamics Corp. of America, New Hartford, Connecticut).
3. Balance: Analytical, Mettler H35AR, precision \pm 0.05 mg.
4. Balance: Pan, Sartorius, Model 2254, precision \pm 5 mg.

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5. Assorted Glassware: General laboratory, flasks, beakers, assorted volumetric flasks, pipets, etc.
6. Microliter Syringe: Hamilton #701-N, 10-mcl capacity.
7. Rotary Evaporator: Buchler Instruments (Model DBL-10GN), equipped with a warm water bath (about 30°C) in which evaporation flasks can be partially submerged.
8. Filtering Funnel: Buchner, Porcelain, 100 mm plate diameter.
9. Filter Paper: 9-cm diameter, glass fiber filter, Whatman, Inc.
10. Recorder: Spectra-physics Model SP 4270 recording integrator.
11. GC Column: 90 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport.
12. Solid Phase Extraction Column: Silica gel, 500 mg., 3-mL (J. T. Baker Chemical Company, Phillipsburg, New Jersey, Cat. No. 7086-3).

C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Analytical Standards: Analytical grade, know purity, American Cyanamid, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.
 - a. Malathion: phosphorodithioic acid, S-,1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
 - b. Malaoxon: phosphorothioic acid, S-1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
2. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Cat. No. 1-1753, Supelco, Incorporated.
3. Solvents: Distilled in Glass, Burdick and Jackson, Incorporated, : acetone, acetonitrile, methylene chloride, and hexane.
4. Activated Carbon: Nuchar C-190N, Cat. No. 5790, Eastman Kodak Co., or equivalent.

D. Preparation of Standard Solutions

Standard Solutions described below are stable for at least one month if kept tightly capped and refrigerated overnight and during periods when they are not being used; allow the solutions to warm to room temperature before opening. The Stock Solutions are stable for at least three months under the same conditions.

1. Stock Solutions

Weigh accurately (to the nearest 0.1 milligram) about 100 milligrams of malathion analytical standard into a glass bottle or flask of about 125-mL capacity with a tightly sealing ground-glass stopper or Teflon-lined screw cap. Fill a 100-mL volumetric flask to the mark with acetone and, using a graduated 5 or 10-mL pipet, add or remove acetone to give a final volume equivalent to 1.0 milliliter of acetone for each milligram of standard weighed. Carefully pour the measured acetone into the bottle containing the analytical standard, cap, and mix well. The resulting stock standard solution contains 1000 mcg/mL malathion. Prepare a 1000-mcg/mL stock standard solution of malaaxon in the same manner.

2. Fortification Solutions

Pipet 10-mL aliquots of each of the stock standard solutions in a single 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution A, contains 100 mcg/mL each of malathion and malaaxon, respectively.

Pipet a 10-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution B, contains 10 mcg/mL each of malathion and malaaxon, respectively.

Pipet a 10-mL aliquot of Solution B into 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution C, contains 1 mcg/mL each of malathion and malaaxon, respectively.

3. Gas Chromatography Standard Solutions

Pipet 50, 25, and 12.5-mL aliquots of Solution C into separate 100-mL volumetric flasks and dilute each to the mark with acetone. These solutions, designated Solutions D, E, and F, contain 0.50, 0.25, and 0.125 mcg/mL, respectively, of each compound. When the analysis is carried out exactly as described, these solutions correspond to residues of 0.20, 0.10 and 0.05 ppm, respectively.

E. Preparation and Conditioning of the Chromatographic Column (Commercial packed columns may be used provided they are functionally equivalent.)

Place a loosely compressed pledget of silanized glass wool in the exit end of the column and attach a funnel to the inlet end by means of a short length of rubber tubing. Pour a small amount of packing into the funnel and tap the column gently to start the flow of packing. Apply gently suction to the exit end of the column and continue tapping the column until the packing is complete. Remove the funnel and vacuum tubing from the column and place a loosely compressed pledget of silanized glass wool in the inlet end of the column to keep the packing in place.

Condition the column in the instrument oven overnight at a temperature about 25°C above the expected operating temperature. In the conditioning step connect the column to the injection port with the normal flow of carrier gas. Do not connect the column to the detector during conditioning. After the conditioning period, connect the column to the detector.

Using as guides the approximate gas chromatographic conditions listed in the next section and the typical chromatograms shown in the attached figures, adjust the instrument to give adequate peak shape, resolution from interfering peaks, and sensitivity such that the malathion peak is about 20% of full-scale deflection when 5-mcL aliquots of Solution F are injected. It may be necessary to make several injections of Solution A and/or a processed sample extract to condition the column. When these conditions have been reached and the responses to the standard compounds are stable to within 10% on repeated injections of Solution F, the instrument is ready for use.

F. Approximate Gas Chromatographic Conditions

Column Temperature	190°C
Inlet Temperature	250°C
Detector Temperature	275°C
Helium Flow Rate	30 mL/min
Hydrogen Flow Rate	100 mL/min
Air Flow Rate	150 mL/min

G. Linearity Check:

A linearity check must be performed just before beginning GC analysis of each batch of processed sample extracts. Inject 5-mcL aliquots of Solutions D, E, and F and plot the peak height for each compound versus its concentration to demonstrate linearity of response. Significant departure from linearity indicates instrumental or operational difficulties which must be corrected before proceeding.

H. Recovery Test

The ability of the analyst to perform these procedures satisfactorily must be demonstrated by recovery tests before analysis of unknown samples is attempted. In addition, at least one recovery sample must be run concurrently with each batch of samples to demonstrate that the overall operation of the procedure for that batch of samples was satisfactory.

Weigh a 20-g portion of untreated sample into a Waring Blender jar and add by pipet a 1-mL aliquot of a fortification solution to yield the desired level. For example, a 1-mL aliquot of the 1-mcg/mL standard added to a 20-gm sample will give a fortification level of 0.05 ppm.

ites. Analyze the sample by the
wing section.

am portion of the sample into a blender
ile and blend for 2 minutes at moderate
ith vacuum through a glass-fiber filter
el. Transfer a 100-mL aliquot of the
ory funnel, add 50 mL of hexane, and
he phases to separate and draw off the
aporation flask. Concentrate the
the rotary evaporator.

1 50 mL of acetone, add 1 g of Nuchar
With the aid of vacuum, filter the
: filter held in a Buchner funnel.
funnel with 50 mL of acetone. Transfer
aporation flask and evaporate to
: silica-gel column by attaching a 10-mL
g 3 mL of a 10% solution of acetone in
ie column.

. 10-mL of 10% acetone in methylene
n through the column, collecting the
g flask. Evaporate to dryness and
or GC analysis. The processed sample
ast two weeks if kept refrigerated and
ration of solvent.

described in Section G just before
each batch of processed sample
L aliquot of each processed sample
aliquot of Solution F. Repeat for each
ate results from the sample and the
jections for a given sample or
10%, one is probably a bad injection;
le-standard injection pair and average
sample peak goes off scale, dilute
ropriate factor with acetone and
factor for use in calculations as

at the injection-port end of the
o malaoxon drops off by 25% or more
tially after equilibration of the
E.

Typical Chromatograms

Corn Fodder
Fortified With
0.05ppm Each

Corn Silage
Fortified With
0.05ppm Each

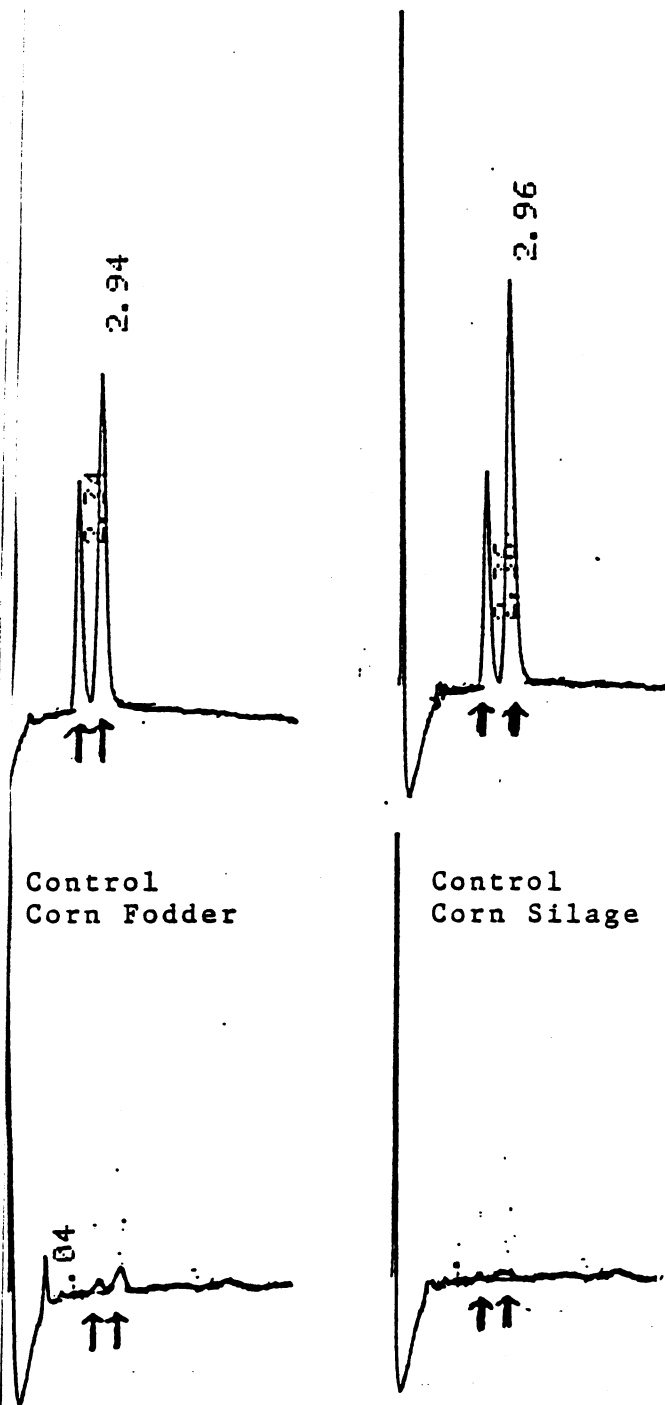
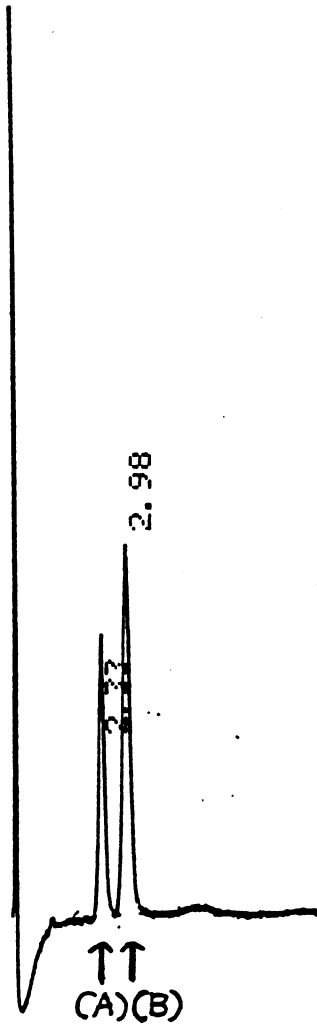


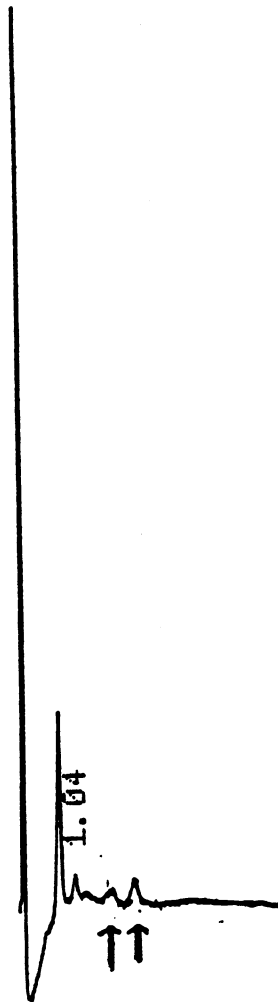
Figure M-1753.B: Typical Chromatograms

Standard
(0.125mcg/mL)

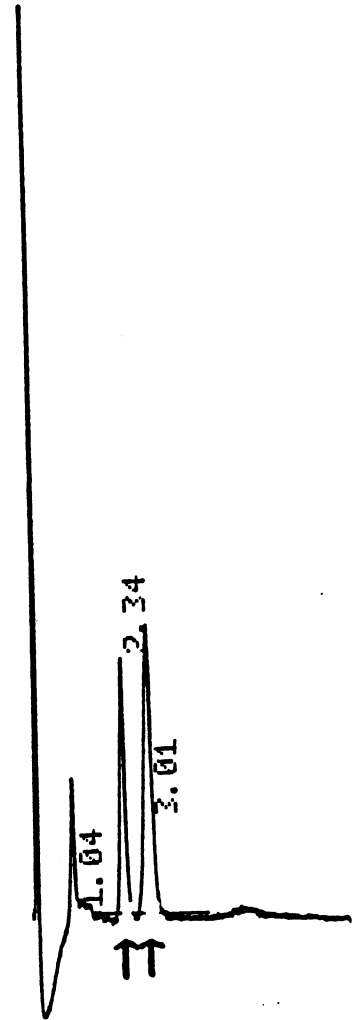
(A) Malaoxon
(B) Malathion



Control Corn
Grain



Corn Grain Fortified
With 0.05ppm Each



OK.



Residue Method M-1782.01
Author D. Kim
D. Kim
Supercedes None
Effective Date 10/26/87
Approved J. Boyd
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Recommended Method of Analysis

CYTHION® malathion (CL 6,601): GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Cotton Seed.

A. Principle

Residues of malathion (CL 6,601) and malaoxon (CL 28,967) are extracted from finely ground plant tissue with acetonitrile. The filtered extracts are subjected to cleanup procedures involving treatment with activated charcoal and passage of a methylene chloride-acetone solution through a disposable silica-gel solid phase extraction cartridge. The malathion (CL 6,601) and malaoxon (CL 28,967) concentrations are determined by gas chromatography using an instrument equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated by direct comparison of peak height to those of external standards. The validated sensitivity of the method is 0.05 ppm for each compound.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
2. Waring Blendor: Model 31BL46 with 1-quart capacity glass blender jar (Waring Products Division, Dynamics Corp. of America, New Hartford, Connecticut).
3. Balance: Analytical, Mettler H35AR, precision \pm 0.05 mg.
4. Balance: Pan, Sartorius, Model 2254, precision \pm 5 mg.

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5. Assorted Glassware: General laboratory, flasks, beakers, assorted volumetric flasks, pipets, etc.
6. Microliter Syringe: Hamilton #701-N, 10-mcl capacity.
7. Rotary Evaporator: Buchler Instruments (Model DBL-10GN), equipped with a warm water bath (about 30°C) in which evaporation flasks can be partially submerged.
8. Filtering Funnel: Buchner, Porcelain, 100 mm plate diameter.
9. Filter Paper: 9-cm diameter, glass fiber filter, Whatman, Inc.
10. Recorder: Spectra-physics Model SP 4270 recording integrator.
11. GC Column: 90 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport.
12. Solid Phase Extraction Column: Silica gel, 500 mg., 3-mL (J. T. Baker Chemical Company, Phillipsburg, New Jersey, Cat. No. 7086-3).

C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Analytical Standards: Analytical grade, know purity, American Cyanamid, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.
 - a. Malathion: phosphorodithioic acid, S-,1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
 - b. Malaaxon: phosphorothioic acid, S-1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
2. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Cat. No. 1-1753, Supelco, Incorporated.
3. Solvents: Distilled in Glass, Burdick and Jackson, Incorporated, : acetone, acetonitrile, methylene chloride, and hexane.
4. Activated Carbon: Nuchar C-190N, Cat. No. 5790, Eastman Kodak Co., or equivalent.

D. Preparation of Standard Solutions

Standard Solutions described below are stable for at least one month if kept tightly capped and refrigerated overnight and during periods when they are not being used; allow the solutions to warm to room temperature before opening. The Stock Solutions are stable for at least three months under the same conditions.

J.K.

1. Stock Solutions

Weigh accurately (to the nearest 0.1 milligram) about 100 milligrams of malathion analytical standard into a glass bottle or flask of about 125-mL capacity with a tightly sealing ground-glass stopper or Teflon-lined screw cap. Fill a 100-mL volumetric flask to the mark with acetone and, using a graduated 5 or 10-mL pipet, add or remove acetone to give a final volume equivalent to 1.0 milliliter of acetone for each milligram of standard weighed. Carefully pour the measured acetone into the bottle containing the analytical standard, cap, and mix well. The resulting stock standard solution contains 1000 mcg/mL malathion. Prepare a 1000-mcg/mL stock standard solution of malaoxon in the same manner.

2. Fortification Solutions

Pipet 10-mL aliquots of each of the stock standard solutions in a single 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution A, contains 100 mcg/mL each of malathion and malaoxon, respectively.

Pipet a 10-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution B, contains 10 mcg/mL each of malathion and malaoxon, respectively.

Pipet a 10-mL aliquot of Solution B into 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution C, contains 1 mcg/mL each of malathion and malaoxon, respectively.

3. Gas Chromatography Standard Solutions

Pipet 50, 25, and 12.5-mL aliquots of Solution C into separate 100-mL volumetric flasks and dilute each to the mark with acetone. These solutions, designated Solutions D, E, and F, contain 0.50, 0.25, and 0.125 mcg/mL, respectively, of each compound. When the analysis is carried out exactly as described, these solutions correspond to residues of 0.20, 0.10 and 0.05 ppm, respectively.

E. Preparation and Conditioning of the Chromatographic Column (Commercial packed columns may be used provided they are functionally equivalent.)

Place a loosely compressed pledget of silanized glass wool in the exit end of the column and attach a funnel to the inlet end by means of a short length of rubber tubing. Pour a small amount of packing into the funnel and tap the column gently to start the flow of packing. Apply gently suction to the exit end of the column and continue tapping the column until the packing is complete. Remove the funnel and vacuum tubing from the column and place a loosely compressed pledget of silanized glass wool in the inlet end of the column to keep the packing in place.

Condition the column in the instrument oven overnight at a temperature about 25°C above the expected operating temperature. In the conditioning step connect the column to the injection port with the normal flow of carrier gas. Do not connect the column to the detector during conditioning. After the conditioning period, connect the column to the detector.

Using as guides the approximate gas chromatographic conditions listed in the next section and the typical chromatograms shown in the attached figure, adjust the instrument to give adequate peak shape, resolution from interfering peaks, and sensitivity such that the malathion peak is about 20% of full-scale deflection when 5-mL aliquots of Solution F are injected. It may be necessary to make several injections of Solution A and/or a processed sample extract to condition the column. When these conditions have been reached and the responses to the standard compounds are stable to within 10% on repeated injections of Solution F, the instrument is ready for use.

F. Approximate Gas Chromatographic Conditions

Column Temperature	190 °C
Inlet Temperature	250 °C
Detector Temperature	275 °C
Helium Flow Rate	30 mL/min
Hydrogen Flow Rate	100 mL/min
Air Flow Rate	150 mL/min

G. Linearity Check:

A linearity check must be performed just before beginning GC analysis of each batch of processed sample extracts. Inject 5-mL aliquots of Solutions D, E, and F and plot the peak height for each compound versus its concentration to demonstrate linearity of response. Significant departure from linearity indicates instrumental or operational difficulties which must be corrected before proceeding.

H. Recovery Test

The ability of the analyst to perform these procedures satisfactorily must be demonstrated by recovery tests before analysis of unknown samples is attempted. In addition, at least one recovery sample must be run concurrently with each batch of samples to demonstrate that the overall operation of the procedure for that batch of samples was satisfactory.

Weigh a 20-g portion of untreated sample into a Waring Blender jar and add by pipet a 1-mL aliquot of a fortification solution to yield the desired level. For example, a 1-mL aliquot of the 1-mcg/mL standard added to a 20-gm sample will give a fortification level of 0.05 ppm.

Let the sample stand for 10 minutes. Analyze the sample by the procedure described in the following section.

I. Sample Handling Procedure

1. Extraction and Partitioning

Weigh a representative 20-gram portion of the sample into a blender jar. Add 200 mL of acetonitrile and blend for 2 minutes at moderate speed. Filter the mixture with vacuum through a glass-fiber filter paper held in a Buchner funnel. Transfer a 100-mL aliquot of the filtrate to a 250-mL separatory funnel, add 50 mL of hexane, and shake for 1 minute. Allow the phases to separate and draw off the lower phase into a 250-mL evaporation flask. Concentrate the solution to near dryness on the rotary evaporator.

2. Cleanup

Dissolve the residual film in 50 mL of acetone, add 1 g of Nuchar activated carbon and shake. With the aid of vacuum, filter the mixture through a glass-fiber filter held in a Buchner funnel. Rinse the flask, filter and funnel with 50 mL of acetone. Transfer the acetone solution to an evaporation flask and evaporate to dryness. Prepare a disposable silica-gel column by attaching a 10-mL disposable syringe and forcing 3 mL of a 10% solution of acetone in methylene chloride through the column.

Dissolve the residual film in 10-mL of 10% acetone in methylene chloride and pass the solution through the column, collecting the eluate in a 100-mL evaporating flask. Evaporate to dryness and dissolve in 4 mL of acetone for GC analysis. The processed sample extracts are stable for at least two weeks if kept refrigerated and tightly capped to avoid evaporation of solvent.

J. Gas Chromatographic Analysis

Perform a linearity check as described in Section G just before beginning the GC analysis of each batch of processed sample extracts. Then inject a 5-mL aliquot of each processed sample solution followed by a 5-mL aliquot of Solution F. Repeat for each sample and average the duplicate results from the sample and the standard. If the duplicate injections for a given sample or standard differ by more than 10%, one is probably a bad injection; in this case make a third sample-standard injection pair and average the two closest results. If a sample peak goes off scale, dilute the sample solution by an appropriate factor with acetone and reinject; record the dilution factor for use in calculations as described below.

Replace the glass-wool pledget at the injection-port end of the column whenever the response to malaoxon drops off by 25% or more from the response obtained initially after equilibration of the column as described in Section E.

D.K

K. Calculations

Calculate the concentration of CL 6,601 or CL 28,967 using the following equation:

$$\text{ppm} = \frac{\text{R(SAMP)} \times \text{V1} \times \text{V3} \times \text{V5} \times \text{C} \times \text{D}}{\text{R(STD)} \times \text{W} \times \text{V2} \times \text{V4}}$$

Where:

R(SAMP) = Average Peak height of sample.

R(STD) = Average peak height of standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extracting solvent in milliliters.

V2 = Volume of extract taken for analysis in milliliters.

V3 = Volume of acetone added to dissolve final residue for chromatographic analysis, in milliliters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of standard solution injected in microliters.

C = Concentration of working standard solution in micrograms per milliliter.

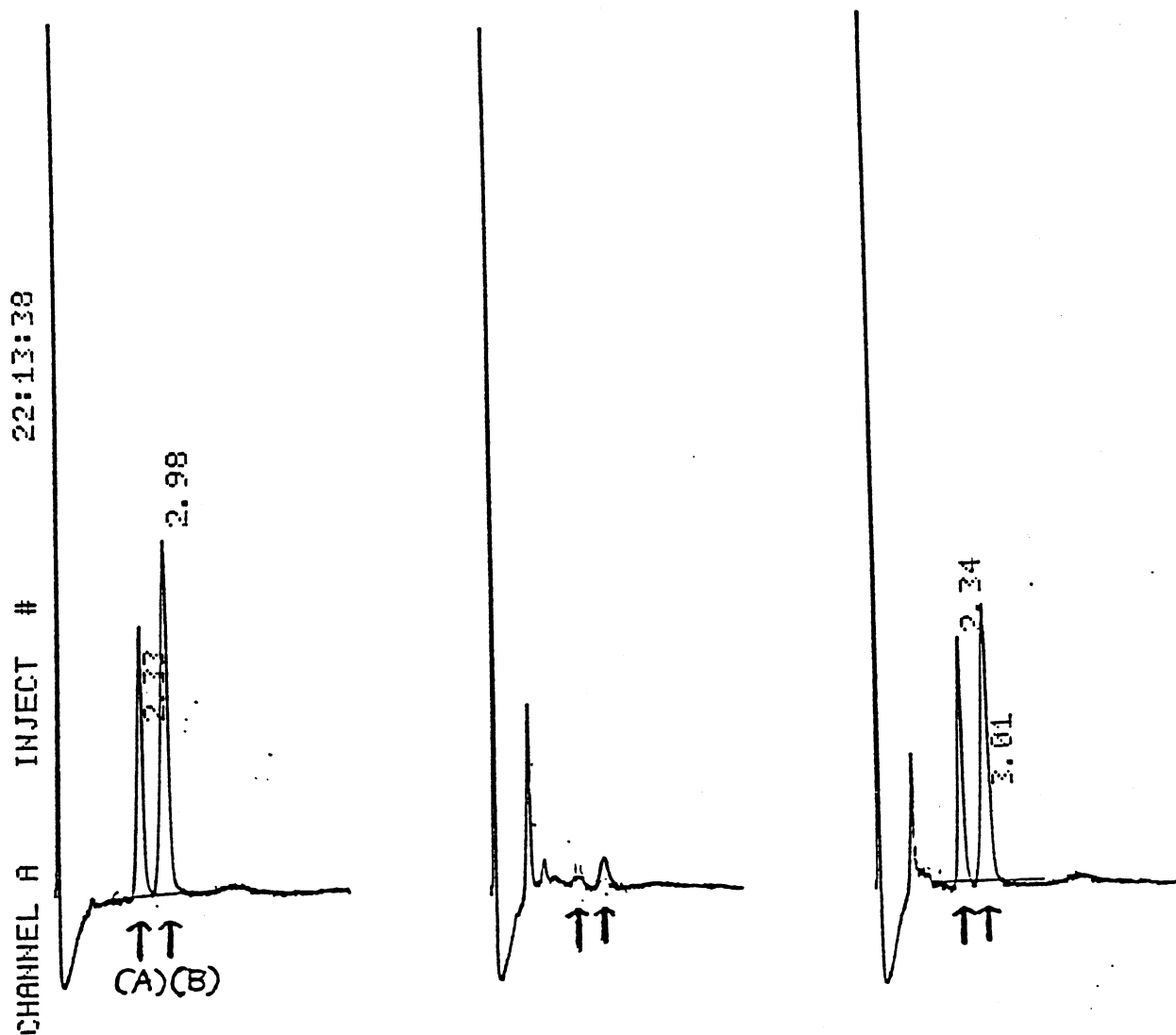
D = Dilution Factor (D=1 if additional dilution is not needed in Section J.

Figure M-1782: Typical Chromatograms

Standard (0.125 mcg/mL)
(A) Malaoxon
(B) Malathion

Control
Cotton Seed

Cotton Seed Fortified
with 0.05 ppm each



Residue Method M-1788
Author D. Kim
D. Kim
Supercedes None
Effective Date 12/07/87
Approved J. Boyd
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Recommended Method of Analysis

CYTHON® malathion (CL 6,601): GC Method for the Determination of Malathion (CL 6,601) and Malaaxon (CL 28,967) Residues in Apples

A. Principle

Residues of malathion (CL 6,601) and malaaxon (CL 28,967) are extracted from finely ground plant tissue with acetonitrile. The filtered extracts are subjected to cleanup procedures involving solvent partitioning and treatment with activated charcoal. The malathion (CL 6,601) and malaaxon (CL 28,967) contents are determined by gas chromatography using an instrument equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated by direct comparison of peak height to those of external standards. The validated sensitivity of the method is 0.05 ppm for each compound.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent)

1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
2. Waring Blendor: Model 31BL46 with 1-quart capacity glass blender jar (Waring Products Division, Dynamics Corp. of America, New Hartford, Connecticut).
3. Balance: Analytical, Mettler H35AR, precision ± 0.05 mg.
4. Balance: Pan, Sartorius, Model 2254, precision ± 5 mg.

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B. 2

5. Assorted Glassware: General laboratory, flasks, beakers, assorted volumetric flasks, pipets, etc.
6. Microliter Syringe: Hamilton #701-N, 10-mcl capacity.
7. Rotary Evaporator: Buchler Instruments (Model DBL-10GN), equipped with a warm water bath about (30°C) in which evaporation flasks can be partially submerged.
8. Filtering Funnel: Buchner, Porcelain, 100 mm plate diameter.
9. Filter Paper: 9-cm diameter, glass fiber filter, Whatman, Inc.
10. Recorder: Spectra-physics Model SP 4270 recording integrator.
11. GC Column: 90 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport.

C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Analytical Standards: Analytical grade, known purity, American Cyanamid, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.
 - a. Malathion: phosphorodithioic acid, S-,1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
 - b. Malaoxon: phosphorothioic acid, S-1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
2. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Cat. No. 1-1753, Supelco, Incorporated.
3. Solvents: Distilled in Glass, Burdick and Jackson, Incorporated, acetone, acetonitrile and methylene chloride.
4. Activated Carbon: Nuchar C-190N, Cat. No. 5790, Eastman Kodak Co., or equivalent.

D. Preparation of Standard Solutions

Standard Solutions described below are stable for at least one month if kept tightly capped and refrigerated overnight and during periods when they are not being used; allow the solutions to warm to room temperature before opening. The Stock Solutions are stable for at least three months under the same conditions.

MP [unclear]

B.K.

1. Stock Solutions

Weigh accurately (to the nearest 0.1 milligram) about 100 milligrams of malathion analytical standard into a glass bottle or flask of about 125-mL capacity with a tightly sealing ground-glass stopper or Teflon-lined screw cap. Fill a 100-mL volumetric flask to the mark with acetone and, using a graduated 5 or 10-mL pipet, add or remove acetone to give a final volume equivalent to 1.0 milliliter of acetone for each milligram of standard weighed. Carefully pour the measured acetone into the bottle continuing the analytical standard, cap, and mix well. The resulting stock standard solution contains 1000 mcg/mL malathion. Prepare a 1000-mcg/mL stock standard solution of malaoxon in the same manner.

2. Fortification Solutions

Pipet 10-mL aliquots of each of the stock standard solutions in a single 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution A, contains 100 mcg/mL each of malathion and malaoxon, respectively.

Pipet a 10-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution B, contains 10 mcg/mL each of malathion and malaoxon, respectively.

Pipet a 10-mL aliquot of Solution B into 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution C, contains 1 mcg/mL each of malathion and malaoxon, respectively.

3. Gas Chromatography Standard Solutions

Pipet 50, 25, and 12.5-mL aliquots of Solution C into separate 100-mL volumetric flasks and dilute each to the mark with acetone. These solutions, designated Solutions D, E, and F, contain 0.50, 0.25, and 0.125 mcg/mL, respectively, of each compound. When the analysis is carried out exactly as described, these solutions correspond to residues of 0.20, 0.10 and 0.05 ppm, respectively.

E. Preparation and Conditioning of the Chromatographic Column (Commercial packed columns may be used provided they are functionally equivalent.)

Place a loosely compressed pledget of silanized glass wool in the exit end of the column and attach a funnel to the inlet end by means of a short length of rubber tubing. Pour a small amount of packing into the funnel and tap the column gently to start the flow of packing. Apply gently suction to the exit end of the column and continue tapping the column until the packing is complete. Remove the funnel and vacuum

tubing from the column and place a loosely compressed pledget of silanized glass wool in the inlet end of the column to keep the packing in place.

Condition the column in the instrument oven overnight at a temperature about 25°C above the expected operating temperature. In the conditioning step connect the column to the injection port with the normal flow of carrier gas. Do not connect the column to the detector during conditioning. After the conditioning period, connect the column to the detector.

Using as guides the approximate gas chromatographic conditions listed in the next section and the typical chromatograms shown in the attached figure, adjust the instrument to give adequate peak shape, resolution from interfering peaks, and sensitivity such that the malaoxon peak is about 20% of full-scale deflection when 5-mL aliquots of Solution F are injected. It may be necessary to make several injections of Solution A and/or a processed sample extract to condition the column. When these conditions have been reached and the responses to the standard compounds are stable to within 10% on repeated injections of Solution F, the instrument is ready for use.

F. Approximate Gas Chromatographic Conditions

Column Temperature	190°C
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B.K.

Weigh a 20-g portion of untreated sample into a Waring Blender jar and add by pipet a 1-mL aliquot of a fortification solution to yield the desired level. For example, a 1-mL aliquot of the 1-mcg/mL standard added to a 20-gm sample will give a fortification level of 0.05 ppm. Let the sample stand for 10 minutes. Analyze the sample by the procedure described in the following section.

I. Sample Handling Procedure

1. Extraction and Partitioning

Weigh a 20-gram portion of the sample into a blender jar. Add 200 mL of acetonitrile and blend for 2 minutes at moderate speed. Filter the mixture with vacuum through a glass-fiber filter paper held in a Buchner funnel. Transfer a 100-mL aliquot of the filtrate to a 500-mL evaporating flask and evaporate the solution to about 50 mL. Transfer the solution to a 250-mL separating funnel and add 50 mL of distilled water. Extract with 50 mL of methylene chloride and re-extract with a fresh 50-mL of methylene chloride. Evaporate the combined extracts to dryness on a rotary evaporator.

2. Cleanup

Dissolve the residual film in 50 mL of acetone, add 1 g of Nuchar activated carbon and shake. With the aid of vacuum, filter the mixture through a glass-fiber filter held in a Buchner funnel. Rinse the flask, filter and funnel with 50 mL of acetone. Transfer the acetone solution to an evaporating flask and evaporate to dryness. Dissolve the residue in 4 mL of acetone for GC analysis.

J. Gas Chromatographic Analysis

Perform a linearity check as described in Section G. just before beginning the GC analysis of each batch of processed sample extracts. Then inject a 5-mL aliquot of each processed sample solution followed by a 5-mL aliquot of Solution F. Repeat for each sample and average the duplicate results from the sample and the standard. If the duplicate injections for a given sample or standard differ by more than 10%, one is probably a bad injection; in this case make a third sample-standard injection pair and average the two closest results. If a sample peak goes off scale, dilute the sample solution by an appropriate factor with acetone and reinject; record the dilution factor for use in calculations as described below. Replace the glass-wool pledget at the injection-port end of the column whenever the response to malaoxon drops off by 25% or more from the response obtained initially after equilibration of the column as described in Section E.

K. Calculations

Calculate the concentration of CL 6,601 or CL 28,967 using the following equation:

$$\text{ppm} = \frac{R(\text{SAMP}) \times V1 \times V3 \times V5 \times C \times D}{R(\text{STD}) \times W \times V2 \times V4}$$

Where:

R(SAMP) = Average Peak height of sample.

R(STD) = Average peak height of standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extracting solvent in milliliters.

V2 = Volume of extract taken for analysis in milliliters.

V3 = Volume of acetone added to dissolve final residue for chromatographic analysis, in milliliters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of standard solution injected in microliters.

C = Concentration of working standard solution in micrograms per milliliter.

D = Dilution Factor (D=1 if additional dilution is not needed in Section J.)

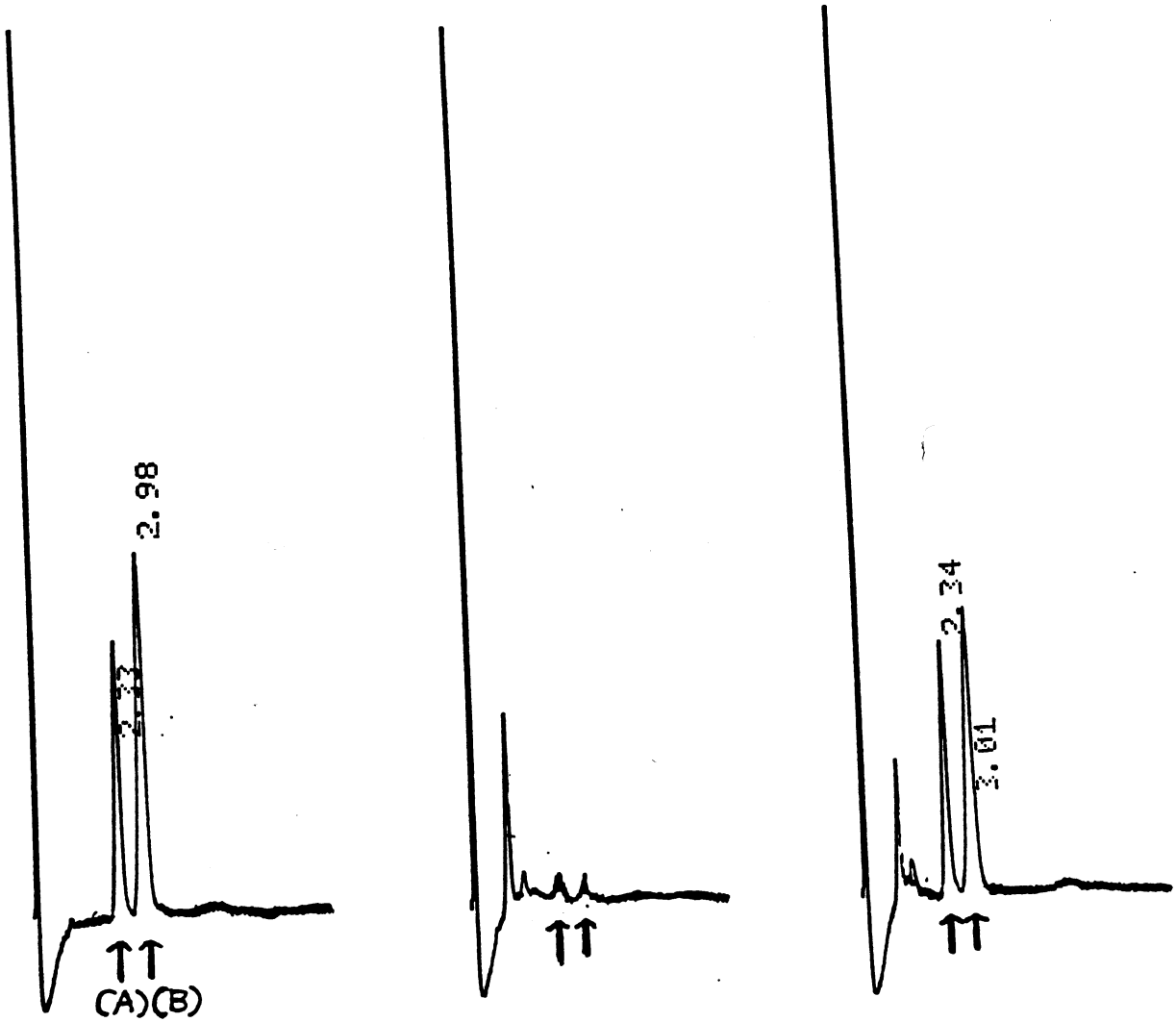
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Figure M-1788: Typical Chromatograms

Standard (0.125 mcg/mL)
(A) Malaoxon
(B) Malathion

Control
Apples

Apples Fortified with
0.05 ppm each



OK



M-1907
D. Kim/hm
03/30/89

Approved by:

John E. Boyd 05/10/8
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Recommended Method of Analysis

CYTHION® malathion (CL 6,601): GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Crude and Refined Oil

A. Principle

Residues of malathion (CL 6,601) and malaoxon (CL 28,967) are extracted from oil in hexane with acetonitrile. The extracts are subjected to cleanup procedures involving treatment with activated charcoal and passage of a methylene chloride-acetone solution through a disposable silica-gel solid phase extraction cartridge. The malathion (CL 6,601) and malaoxon (CL 28,967) concentrations are determined by gas chromatography using an instrument equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated by direct comparison of peak height to those of external standards. The validated sensitivity of the method is 0.05 ppm for each compound.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
2. Balance: Analytical, Mettler H35AR, precision \pm 0.05 mg.
3. Balance: Pan, Sartorius, Model 2254, precision \pm 5 mg.
4. Assorted Glassware: General laboratory, flasks, beakers, assorted volumetric flasks, pipets, etc.

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5. Microliter Syringe: Hamilton #701-N, 10-mcL capacity.
 6. Rotary Evaporator: Buchler Instruments (Model DBL-10GN), equipped with a warm water bath (about 30°C in which evaporation flasks can be partially submerged).
 7. Filtering Funnel: Buchner, Porcelain, 100 mm plate diameter.
 8. Filter Paper: 9-cm diameter, glass fiber filter, Whatman, Incorporated.
 9. Recorder: Spectra-physics Model SP 4270 recording integrator.
 10. GC Column: 90 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport.
 11. Solid Phase Extraction Column: Silica gel, 500 mg, 3-mL (J. T. Baker Chemical Company, Phillipsburg, New Jersey, Cat. No. 7086-3).
- C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent).
1. Analytical Standard: Analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.
 - a. Malathion: phosphorodithioic acid, S-,1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
 - b. Malaaxon: phosphorothioic acid, S-,1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
 2. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Cat No. 1-1753, Supelco, Incorporated.
 3. Solvents: Distilled in Glass, Burdick and Jackson, Incorporated, : acetone, acetonitrile, methylene chloride, and hexane.
 4. Activated Carbon: Nuchar C-190N, Cat. No. 5790, Eastman Kodak Company, or equivalent.
 5. Polyethylene Glycol, 400: P-165, Fisher Scientific Company, Fair Lawn, New Jersey 07410.
 6. Acetone-PEG: 0.02% PEG in acetone, 200 mcL of polyethylene glycol 400 was added to 1,000 mL of acetone.

D. Preparation of Standard Solutions

Standard solutions described below are stable for at least one month if kept tightly capped and refrigerated overnight and during periods when they are not being used; allow the solutions to warm to room temperature before opening. The Stock Solutions are stable for at least three months under the same conditions.

1. Stock Solutions

Weigh accurately (to the nearest 0.1 milligram) about 100 milligrams of malathion analytical standard into a glass bottle or flask of about 125-mL capacity with a tightly sealing ground-glass stopper or Teflon-lined screw cap. Fill a 100-mL volumetric flask to the mark with acetone and, using a graduated 5 or 10-mL pipet, add or remove acetone to give a final volume equivalent to 1.0 milliliter of acetone for each milligram of standard weighed. Carefully pour the measured acetone into the bottle containing the analytical standard, cap, and mix well. The resulting stock standard solution contains 1000 mcg/mL malathion. Prepare a 1000-mcg/mL stock standard solution of malaaxon in the same manner.

2. Fortification Solutions

Pipet 10-mL aliquots of each of the stock standard solutions in a single 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution A, contains 100 mcg/mL each of malathion and malaaxon, respectively.

Pipet a 10-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution B, contains 10 mcg/mL each of malathion and malaaxon, respectively.

Pipet a 10-mL aliquot of Solution B into 100-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution C, contains 1 mcg/mL each of malathion and malaaxon, respectively.

3. Gas Chromatography Standard Solutions

Pipet 50, 25, and 12.5-mL aliquots of Solution C into separate 100-mL volumetric flasks and dilute each to the mark with acetone. These solutions, designated Solutions D, E, and F, contain 0.50, 0.25, and 0.125 mcg/mL, respectively, of each compound. When the analysis is carried out exactly as described, these solutions correspond to residues of 0.20, 0.10 and 0.05 ppm, respectively.

E. Preparation and Conditioning of the Chromatographic Column (Commercial packed columns may be used provided they are functionally equivalent.)

Place a loosely compressed pledget of silanized glass wool in the exit end of the column and attach a funnel to the inlet end by means of a short length of rubber tubing. Pour a small amount of packing into the funnel and tap the column gently to start the flow of packing. Apply gentle suction to the exit end of the column and continue tapping the column until the packing is complete. Remove the funnel and vacuum tubing from the column and do not place glass wool in the inlet end of the column.

Condition the column in the instrument oven overnight at a temperature about 25°C above the expected operating temperature. In the conditioning step connect the column to the injection port with the normal flow of carrier gas. Do not connect the column to the detector during conditioning. After the conditioning period, connect the column to the detector.

Using as guides the approximate gas chromatographic conditions listed in the next section and the typical chromatograms shown in the attached figure, adjust the instrument to give adequate peak shape, resolution from interfering peaks, and sensitivity such that the malathion peak is about 20% of full-scale deflection when 5-mL aliquots of Solution F are injected. It may be necessary to make several injections of Solution A and/or a processed sample extract to condition the column. When these conditions have been reached and the responses to the standard compounds are stable to within 10% on repeated injections of Solution F, the instrument is ready for use.

F. Approximate Gas Chromatographic Conditions

1. Column Temperature	190°C
2. Inlet Temperature	250°C
3. Detector Temperature	275°C
4. Helium Flow Rate	30 mL/min
5. Hydrogen Flow Rate	100 mL/min
6. Air Flow Rate	150 mL/min

G. Linearity Check

A linearity check must be performed just before beginning GC analysis of each batch of processed sample extracts. Inject 5-mL aliquots of Solutions D, E, and F and plot the peak height for each compound versus its concentration to demonstrate linearity of response. Significant departure from linearity indicates instrumental or operational difficulties which must be corrected before proceeding.

H. Recovery Test

The ability of the analyst to perform these procedures satisfactorily must be demonstrated by recovery tests before analysis of unknown samples is attempted. In addition, at least one recovery sample must be run concurrently with each batch of samples to demonstrate that the overall operation of the procedure for that batch of samples was satisfactory.

Weigh a 10-g portion of untreated sample into 250-mL separatory funnel add by pipet a 0.5-mL aliquot of a fortification solution to yield the desired level. For example, a 0.5-mL aliquot of the 1-mcg/mL standard added to a 10-gm sample will give a fortification level of 0.05 ppm. Let the sample stand for 1 minute. Analyze the sample by the procedure described in the following section.

I. Sample Handling Procedure

1. Extraction and Partitioning

Weigh a representative 10-gram portion of the sample into a small beaker and transfer to a 250-mL separatory funnel with 50 mL of hexane. Add 50 mL of acetonitrile, and shake for 1 minute. Allow the phases to separate and draw off the lower phase into 250-mL evaporating flask. Extract two more times with 50 mL of acetonitrile and combine in a 500-mL evaporating flask. Concentrate the solution to near dryness on the rotary evaporator.

2. Dissolve the residual film in 50 mL of acetone, add 1 g of Nuchar activated carbon and shake. With the aid of vacuum, filter the mixture through a glass-fiber held in a Buchner funnel. Rinse the flask, filter and funnel with 50 mL of acetone. Transfer the acetone solution to an evaporation flask and evaporate to dryness. Prepare a disposable silica-gel column by attaching a 10-mL disposable syringe and forcing 3 mL of a 10% solution of acetone in methylene chloride through the column.

Dissolve the residual film in 10-mL of 10% acetone in methylene chloride and pass the solution through the column, collecting the eluate in a 100-mL evaporating flask. Evaporate to dryness and dissolve in 4 mL of acetone for GC analysis. The processed sample extracts are stable for at least two weeks if kept refrigerated and tightly capped to avoid evaporation of solvent.

J. Gas Chromatographic Analysis

Perform a linearity check as described in Section G just before beginning the GC analysis of each batch of processed sample extracts. Then inject a 5-mcL aliquot of each processed sample solution followed by a 5-mcL aliquot of Solution F. Repeat for each sample and average the duplicate results from the sample and the standard. If the duplicate injections for a given sample or standard differ by more than 10%, one is probably a bad injection; in this case make a third sample-standard injection pair and average the two closest results. If a sample peak goes off scale, dilute the sample solution by an appropriate factor with acetone and reinject; record the dilution factor for use in calculations as described below.

Replace the column packing at the injection-port end of the column whenever the response to malaoxon drops off by 25% or more from the response obtained initially after equilibration of the column as described in Section E.

K. Calculations

Calculate the concentration of CL 6,601 or CL 28,967 using the following equation:

$$\text{ppm} = \frac{R(\text{SAMP}) \times V1 \times V3 \times V5 \times C \times DF}{R(\text{STD}) \times W \times V2 \times V4}$$

Where:

R(SAMP) = Average Peak height of sample.

R(STD) = Average Peak height of standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extracting solvent in milliliters.

V2 = Volume of extract taken for analysis in milliliters.

V3 = Volume of acetone added to dissolve final residue for chromatographic analysis, in milliliters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of standard solution injected in microliters.

C = Concentration of working standard solution in micrograms per milliliter.

DF = Dilution Factor (DF=1 if additional dilution is not needed in Section J).

Figure M-1907: Typical Chromatograms

