1.0 INTRODUCTION

An independent method validation study was conducted at PTRL West, Inc. (625-B Alfred Nobel Dr, Hercules, California) to determine the validity of a procedure to analyze Malathion and Malaoxon in Soil. See Appendix A for the study Protocol and Protocol Amendment. The study was initiated on August 24th, 2011. The independent laboratory validation was conducted from August 24, 2011 through August 31, 2011.

2.0 MATERIALS AND METHODS

2.1 Method

The analytical method for the analysis of Malathion and Malaoxon in Soil was conducted relative to a method validated at Morse Laboratories (Reference 1, See Also Appendix A-Protocol Appendix 1).

The determination of Malathion and Malaoxon was validated by spiking known concentrations of each analyte into control soil samples. An acetonitrile extraction of the analytes from the matrix is performed followed by centrifugation to remove the solids. A portion of the combined extract is then acidified and applied to a pre-conditioned OASIS HLB cartridge. The cartridge is washed successively with ammonium hydroxide/5% methanol and acetic acid/5% methanol. The analytes are then eluted with methanol. The eluent is concentrated and then diluted with water/0.088% formic acid and submitted for HPLC/MS/MS analyses in the positive mode. The percent recovery was determined relative to an external calibration curve.

2.2 Test System

The test system was soil. The control soil sample used for this study had an identification number of MSL-PF 4-8" and was received from Agvise Laboratories, Inc. on August 10, 2011. The soil sample originated from North Dakota (Agvise Site name : MSL-PF 4-8"). Soil Characterization report is provided in Appendix C.

2.3 Reference Substances

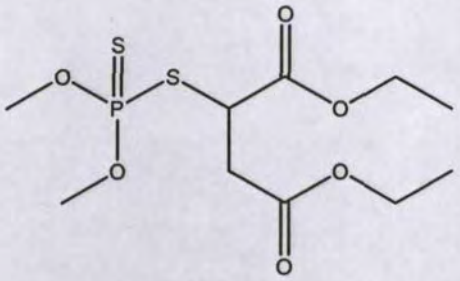
The Malathion (1999W-003), and Malaoxon (2144W-001) reference substances were obtained from Cheminova, A/S. Stock solutions of the reference substances were prepared at 1.0 mg/mL in acetonitrile. Calibrant solutions appeared stable when stored

refrigerated for at least 6 days, based on the comparison of LC-MS/MS chromatograms during this study. The certificates of analysis for the reference substances are provided in Appendix B.

Compound: **Malathion**

Chemical Name: Butanedioic acid, [(dimethoxyphosphinothioyl)-thio]-, diethyl ester

Chemical Structure:

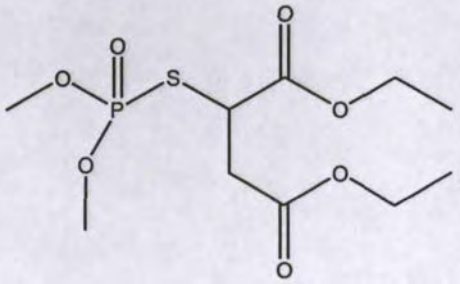


Purity: 99.6%
Lot Number: 650-OSJ-36E
PTRL West Inventory Number: 1999W-003
Date Received: July 16, 2010
Expiration Date: March 1, 2018
Storage Conditions: Freezer

Compound: **Malaoxon**

Chemical Name: Butanedioic acid, [(dimethoxyphosphinyl)-thio]-, diethylester]

Chemical Structure:



Purity: 96.2%
Lot Number: 849-Bse-39B
PTRL West Inventory Number: 2144W-001
Date Received: May 11, 2011
Expiration Date: October 6, 2014

Storage Conditions: Freezer

2.4 Equipment

Balance (various types)
Bottle, amber glass, 120 mL
Centrifuge bottle, HDPE, 250 mL
Centrifuge (Sorvall RT7 plus)
Graduated cylinder, various sizes
Glass tubes, 50 mL Pyrex
Glass disposable centrifuge tubes 15 mL
Pasteur pipettes
Sonicator, Branson 2210
SPE cartridges Oasis HLB (60 mg, 3 cc)
Syringes, microliter, various sizes
Vacuum evaporator, (Zymark TurboVap LV evaporator)
Vacuum manifold with stopcocks for SPE cartridges
Volumetric flask, various sizes
Vacuum pump
Wrist-action shaker

2.5 Solvents and Reagents

All solvents and reagents (reagent grade or better) were obtained from Fisher Scientific or VWR. Ammonium hydroxide was obtained from Sigma Aldrich. All water used was HPLC grade.

Acetic acid
Methanol
Acetonitrile
Ethyl Acetate
Water
Formic Acid
Ammonium hydroxide

2.6 Reference Substance Stock Solution Preparation

Stock standard solutions of malathion and malaoxon (corrected for purity) were prepared at approximately 1.0 mg/mL in acetonitrile. The stock standards were stored frozen when not in use. These solutions were stable for the period of the study based on comparison of chromatograms during the study duration.

2.7 Preparation of Intermediate/Fortification Solutions

Individual solutions were prepared as follows:

100.0 µg/mL: 2.5 ml of each 1000 µg/mL stock solution was transferred into individual 25 ml volumetric flasks, diluted to the mark with acetonitrile and mixed well.

10.0 µg/mL: 2.5 ml of each 100 µg/mL solution was transferred into individual 25 ml volumetric flasks, diluted to the mark with acetonitrile and mixed well.

1.0 µg/mL: 2.5 ml of each 10 µg/mL stock solution was transferred into individual 25 ml volumetric flasks, diluted to the mark with acetonitrile and mixed well.

Mixed solutions were prepared as follows:

100.0 µg/mL: 1.0 ml of each 1000 µg/mL stock solution was transferred into a single 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.

10.0 µg/mL: 1.0 ml of each 100 µg/mL stock solution was transferred into a single 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.

1.0 µg/mL: 1.0 ml of each 10 µg/mL stock solution was transferred into a single 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.

0.1 µg/mL: 1.0 ml of each 1.0 µg/mL stock solution was transferred into a single 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.

Intermediate/Fortification solutions were transferred to the amber grass bottles and stored frozen when not in use.

2.8 Preparation of Calibrants

Calibration standard solutions were prepared as follows:

- 2.5 ng/mL: 250 µL of the 0.1 µg/mL intermediate mixed solution was transferred into a 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.
- 0.5 ng/mL: 50 µL of the 0.1 µg/mL intermediate mixed solution was transferred into a 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.
- 0.25 ng/mL: 25 µL of the 0.1 µg/mL intermediate mixed solution was transferred into a 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.
- 0.1 ng/mL: 400 µL of the 2.5 ng/mL mixed standard solution was transferred into a 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.
- 0.05 ng/mL: 200 µL of the 2.5 ng/mL mixed standard solution was transferred into a 10 ml volumetric flask, diluted to the mark with acetonitrile and mixed well.

Standards were mixed well in volumetric flasks, then transferred to amber glass bottles. All calibration solutions were stored in the refrigerator when not in use.

2.9 Sample Preparation

The control soil sample was received from Agvise Laboratories, Inc. on August 10, 2011. The soil sample originated from North Dakota (Agvise Site name : MSL-PF 4-8").

Aliquots of the soil (10.0 g) were weighed into 250 mL HDPE centrifuge bottles prior to fortification (if necessary) and extraction.

2.10 Sample Fortification

Fortification of control soil was performed to analyze method percent recoveries for the ILV. A portion (10 g of soil) was fortified with 100 µL of the listed Fortification solution as follows:

Fortification Designation	Fortification Level (mg/kg)	Concentration of Fortification solution used
F1A	0.01 (malathion and malaoxon each)	1.0 µg/mL

F1B	0.01 (malathion and malaoxon each)	1.0 µg/mL
F1C	0.01 (malathion and malaoxon each)	1.0 µg/mL
F1D	0.01 (malathion and malaoxon each)	1.0 µg/mL
F1E	0.01 (malathion and malaoxon each)	1.0 µg/mL
F2A	0.1 (malathion and malaoxon each)	10.0 µg/mL
F2B	0.1 (malathion and malaoxon each)	10.0 µg/mL
F2C	0.1 (malathion and malaoxon each)	10.0 µg/mL
F2D	0.1 (malathion and malaoxon each)	10.0 µg/mL
F2E	0.1 (malathion and malaoxon each)	10.0 µg/mL

2.11 Extraction Method

1. Weigh 10.0 g of homogenized soil sample into a 250 mL HDPE centrifuge bottle. Fortify as described in Section 2.10. The 100 µL of fortification solution is added to the surface of the soil using a Hamilton syringe.

2. Add 100 mL of acetonitrile. Cap and shake on a platform shaker at a medium speed (180 excursions per minute) shaker for 30 minutes.

3. Centrifuge at 3,000 rotations/minute for 10 minutes. Decant the supernatant into a 500 mL glass graduated mixing cylinder.

4. Repeat the extraction with 100 mL of acetonitrile two more times (see steps 2 and 3), combining supernatants in the same 500 mL cylinder.

5. Adjust the volume of the extract to 500 mL with acetonitrile. Mix well.

6. Transfer 30 ml to a 50 ml graduated polypropylene centrifuge tube and cap. Mix well. Centrifuge if necessary at 3000 rpm for 10 minutes. Remove aliquots for analyses as specified in Step 2.11.7 and store remainder at 1-8 °C as a retain extract of the sample as needed for reanalyses.

7. Transfer 1.0 mL of the extract from Step 2.11.6 to a 15 mL disposable centrifuge tube containing 9.0 ml of 0.088% formic acid in DI water. Cap and Vortex-mix.

8. OASIS HLB SPE Cartridge Cleanup

i. Set up an SPE cleanup system with Oasis HLB 60 mg, 3cc cartridge on a vacuum manifold connected to a vacuum pump. In general, set vacuum to produce a flow rate of approximately 2 ml/minute (not continuous flow) for all elutions.

ii. Condition an Oasis HLB cartridge (size 3 cc, 60mg) by passing 2 mL of methanol followed by 2 ml of deionized water, through the cartridge. Do not let the cartridge become dry in between any of the conditioning steps or between the conditioning and sample introduction. (Stop elution when each conditioning solvent reaches the top of the frit.) Discard all eluates.

iii. Pass the sample extract (10 ml) from Step 2.11.7 through the SPE cartridge. Stop elution when the solvent reaches the top of the frit. Discard the eluate. Residues of malathion and malaoxon are retained on the cartridge.

iv. Add 1 ml of 5% methanol in DI water to the empty centrifuge tube that contained the sample extract. Shake to rinse the tube and pass the rinse through the cartridge. Stop elution when the solvent reaches top of frit. Discard eluate.

v. Wash the sample-laden cartridge with 1 ml of ammonium hydroxide:5% methanol in DI water (2:98, v/v). Stop elution when the solvent reaches top of frit. Discard eluate.

vi. Wash the sample laden cartridge with 1 ml of acetic acid:5% methanol in DI water (2:98, v/v). Stop elution when the solvent reaches top of frit. Discard eluate.

vii. Place a 15 ml polypropylene centrifuge tube calibrated at 1.0 and 2.0 ml under the SPE cartridge

viii. Elute the analytes with 2 mL of HPLC methanol into the calibrated 15 mL polypropylene tube. Allow the cartridge to dry under vacuum.

ix. Using an N-Evap with the water bath set at 35 °C concentrate the eluate to the 1.0 ml calibrated mark.

x. Bring to a final volume of 2.0 ml with 0.088% formic acid in HPLC water. Sonicate for 1 minute and vortex for 30 seconds to mix well.. Submit for LC-MS/MS analyses.

2.12 LC-MS/MS Analysis of malathion and malaoxon

An Applied Biosystems MDS/SCIEX API 4000 LC/MS/MS system with electrospray ionization and an Agilent 1100 series LC system were used.

2.12.1 LC System Components

SCIEX 4000 (HPLC/Turbo Ion Spray Mode)

Pump: Agilent 1100 series, model G1312A

Autosampler: Agilent 1100 series, model G1329A

Micro-Degasser: Agilent 1100 series, model G1379A

Column Compartment: Agilent 1100 series, model G1316A

2.12.2 LC Parameters

Column: Phenomenex Synergi Fusion RP, 100A (100 mm x 2.0 mm I.D.), plus a 4 x 2 mm Phenomenex Fusion security guard pre column cartridge.
Flow Rate: 200 µL/minute
Injection Volume: 10 µL
Temperature: 40°C
Solvent System: Solvent A = Water (0.1% formic acid)
Solvent B = Acetonitrile

Solvent Program:

Minutes	Solvent A	Solvent B
0	75	25
0.5	75	25
5.0	0	100
9.0	0	100
9.5	75	25
13.0	75	25

Divert Valve: Divert LC flow from column to waste (bypassing MS) from 0 to 6.0 minutes and again from 9.0 to 13.0 minutes.

Approximate Retention Times: malaoxon: 7.1 minutes, malathion: 8.6 minutes,

2.12.3 Mass Spectrometer Parameters

An Applied Biosystems MDS/SCIEX API 4000 LC/MS/MS system was used with electrospray ionization in positive polarity mode to acquire data by Multiple Reaction Monitoring (MRM):

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (msec)	Declustering Potential	Collision Energy	Collision Activation Dissociation Gas	Collision Cell Exit Potential
315.2	126.9	150	40	18	6	11.0
315.2	99.1	150	40	35	6	7.0
315.2	143.1	150	40	16	6	12.0
331.1	127.0	150	40	19	6	11.0
331.1	99.1	150	40	33	6	8.0
331.1	285.3	150	40	11.5	6	7.0

Source Dependent Settings:

Nebulizer Temperature (TEM):	350°C
Nebulizer Gas (GS1):	40
Turbo Gas (GS2):	80
Ion Spray Voltage (IS):	5500
Curtain Gas (CUR):	10
Interface Heater (ihe):	on
Entrance potential:	10

Separation of the analytes was achieved by high performance liquid chromatography. The analyte was identified by the coincidence of it retention time with that of the respective reference standards as well as by monitoring specific ion transitions.

3.0 METHODS OF CALCULATION

3.1 Preparation of Stock Standards

$$\text{Final Concentration (mg/mL)} = \frac{(W) \times (P)}{(VS)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
VS = Volume of Solvent (mL)

3.2 Residue in Matrices

Each analyte was quantified by peak area relative to an external calibration curve. A calibrant peak area (y) from the quantitation ion transition relative to the concentration of the calibrant in ng/mL (x) yielded a linearity curve, where $y = mx + b$ was plotted using a least squares fit with 1/x weighting. Curves are determined by Applied Biosystems/MDS SCIEX Instruments Analyst Software version 1.4.2 or the equivalent

The residue of each analyte (malathion and malaoxon) in soil was calculated as follows:

$$\text{mg/kg (ppm)} = \frac{\text{ng/mL analyte} \times \text{Final vol.(mL)} \times \text{Extraction volume(ml)} \times 0.001\ \mu\text{g/ng} \times \text{Dil factor}}{\text{Sample Wt.(g)} \times \text{Aliquot volume (ml)}}$$

Where:

ng/mL analyte	=	ng/mL of analyte found from standard curve
Final vol. (mL)	=	Volume of final HPLC ready extract (2 mL)
Extraction volume	=	Volume of extraction solvent (500 ml)
Dil Factor	=	1.0 (no dilution of analyses samples performed)
0.001 µg/ng	=	Unit conversion factor
Sample weight	=	10.0 grams
Aliquot volume	=	Volume of extract taken through Oasis HLB SPE cleanup (1.0 ml)

And:

ng/mL analyte	=	[(PA – b) ÷ m]

Where:

PA	=	Peak area analyte
b	=	y-intercept of calibration curve
m	=	slope of calibration curve

$$\% \text{ Recovery} = \frac{\text{Analyte Residue Detected (mg/kg) - Avg. Control Residue}}{\text{Analyte Fortification Level (mg/kg)}} \times 100$$

An example calculation for the malathion residue in soil (Fort 1A at 0.01 mg/kg) is shown below:

Linear regression analysis (with 1/x weighting) of the malathion standards gave a curve as calculated by the Analyst Software version for the quantitation ion transition 331.1/285.3 with the equation

$$y = 245167.705\ x + 7008.8117633\ (r^2 = 0.999778723354)$$

The ng/mL malathion injected determined by this curve was:

ng/mL malathion = [((28928.26665 - 7008.8117633) ÷ 245167.705 = 0.0894 ng/mL

Where:

28928.26665 = peak area malathion
7008.8117633 = y-intercept of calibration curve
245167.705 = slope

The malathion residue (mg/kg)

= (0.0894 ng/mL x 2.0 mL x 500 ml x 1.0 x 0.001 µg/ng) ÷ (10 g x 1.0 ml)

= 0.00894 µg/g or mg/kg

$$\text{Percent Recovery} = \frac{0.00894\text{mg/kg} - 0.00000\ \text{mg/kg}}{0.01\,\text{mg/kg}} \times 100 = 89\%$$

4.0 LIMIT OF QUANTITATION AND DETECTION

The limit of detection (LOD) is defined as the concentration of the lowest linearity calibrant injected – 0.05 ng/ml malathion and malaoxon. Using the current methodology this is equivalent to an LOD of 0.005 ppm for both analytes. The limit of quantitation (LOQ) is defined as the lowest concentration validated which is 0.01 ppm for malathion and malaoxon.

5.0 STATISTICAL METHODS

The residue data included the following statistical calculations: averages, standard deviations, relative standard deviation and linear regression analysis with 1/x weighting.

6.0 TIME REQUIRED FOR ANALYSIS

A sample set consisting of 12 samples and a reagent blank sample can be completed by one analyst in the following amount of time:

Extraction/Cleanup: 14 hrs.

Analysis: 13 hr. for analysis (includes 4 hours for instrument tuning).

Total person-hours = 16 (does not include 7 hrs of unattended LC-MS/MS analysis or 4 hrs for tuning)

Days Required for the Sample Set = 2 Days

7.0 ARCHIVING STATEMENT

The original project specific data files will be stored at PTRL-West (Hercules CA). The project specific data files will be transferred to a location specified by the Sponsor upon their authorization. No data will be discarded without the Sponsor's written consent. Facility records and a copy of the final report are to be maintained at PTRL West. The test system will be retained at PTRL West. Following Sponsor's authorization, the test system may be sent to the Sponsor or disposed of, in accordance with PTRL West standard operating procedures.

DETERMINATION OF MALATHION AND MALAOXON IN SOIL BY LC-MS/MS

Reason for Revision: To correct the title of the original version, dated May 25, 2011, to read as initially intended (adding "by LC-MS/MS").

1 PRINCIPLE

The method described herein is capable of determining malathion and malaoxon in soil.

Residues of malathion and malaoxon are extracted from the sample with acetonitrile using multiple extractions (3 extractions). Following each centrifugation, the crude extract (supernatant) is decanted and combined into a graduated cylinder. The combined extracts are brought to a final known volume, and then mixed thoroughly. An aliquot of the combined extract is purified by means of an Oasis® HLB solid phase extraction (SPE) cleanup. The purified extract is submitted in HPLC methanol:0.088% formic acid (50:50, v/v) for HPLC analysis.

During routine analysis, determination and quantitation for malathion and malaoxon are conducted using HPLC employing mass spectrometric (MS/MS) detection (LC/MS/MS). The limit of quantitation (LOQ) is 0.01 ppm for malathion and malaoxon.

2 EQUIVALENCE STATEMENT

During the conduct of this analysis, comparable apparatus, solvents, glassware, and techniques (such as sample extract evaporation) may be substituted for those described in this method, except where specifically specified. In the event a substituted piece of equipment or technique is used, its use will be documented in the study records.

3 APPARATUS AND EQUIPMENT

Assorted laboratory glassware

Balances:	Analytical balance capable of weighing to ±0.1 mg
	Top-loading balance capable of weighing to ±0.01 g
Centrifuges:	Centrific™ Centrifuge (Fisher Scientific, Fairlawn, NJ)
	IEC Clinical centrifuge (International Equipment Co., Needham Heights, MA)
Evaporator:	N-Evap Laboratory Sample Evaporator Model 115 attached to a N_2 source (Organomation Assoc., South Berlin, MA)
Extract storage containers:	Polypropylene (PP), graduated; 50-mL

Extraction vessels:	HDPE centrifuge bottles; 250-mL (VWR Scientific, Bridgeport, NJ)
Graduated cylinders:	Glass; various sizes
Graduated mixing cylinders:	Glass; various sizes
LC/MS/MS system:	Applied Biosystems/Sciex API 4000 LC/MS/MS System with ACQUITY UPLC System including Sample Organizer with Applied Biosystems/MDS Sciex Analyst Software for data collection and system control (version 1.5)
Microliter syringes:	Various sizes (Hamilton Co., Reno, NV)
Pasteur pipets:	Glass, 9-inch and 5½-inch, disposable
Pipets:	Glass, graduated, serological; various sizes
Pipets, adjustable:	Finnpipette digital pipettors: 40-200 µL: VWR Scientific Catalog #53515-052 100-1000 µL: Fisher Catalog #14-386-74 Finnpipette pipet tips: 1-200 µL: VWR Scientific Catalog #53508-810 100-1000 µL: VWR Scientific Catalog #53516-164 Eppendorf Research Series 2100: 1-10 mL: Eppendorf Catalog # 022472208
Platform shaker:	Eberbach Model 6000 (Eberbach Corp., Ann Arbor, MI)
Solid Phase Extraction Apparatus:	Visiprep 12 or 24-port SPE vacuum manifold with disposable flow control liners (Supelco, Bellefonte, PA)
Standard bottles:	Glass, amber; various sizes
Ultrasonic bath:	Branson Model 2210 ultrasonic bath (VWR Scientific, Bridgeport, NJ)
Volumetric flasks:	Glass; various sizes

4 REAGENTS AND MATERIALS

Acetic acid:	Glacial, 100%, HPLC grade, (Fisher Scientific, Fairlawn, NJ)
Acetonitrile:	Optima® (Fisher Scientific, Fairlawn, NJ)
	HPLC grade (Fisher Scientific, Fairlawn, NJ)
Ammonium hydroxide:	28-30% GR ACS (EMD Chemicals, Gibbstown, NJ)
Formic acid:	88%, certified, A.C.S. (Fisher Scientific, Fairlawn, NJ)
	98% GR ACS (EMD Chemicals, Gibbstown, NJ)
HPLC column:	100 mm × 2.0 mm i.d. Phenomenex Luma C18(2)-HST, 2.5µ particle size
Methanol:	Optima® (Fisher Scientific, Fairlawn, NJ)
	HPLC grade (Fisher Scientific, Fairlawn, NJ)
Reference standards:	Malathion: Analytical grade
	Malaoxon: Analytical grade
Solid phase extraction cartridges:	Oasis® HLB extraction cartridges; 3cc, 60 mg (Waters Corporation, Milford, MA; Catalog #WAT094226)
Water:	Deionized (DI) water (Polymetrics System, Morse Laboratories)
	HPLC grade (Fisher Scientific, Fair Lawn, NJ)

4.1 Reagents and Materials to be Prepared (including typical preparation instructions)

4.1.1 *0.088% formic acid in HPLC grade water:* Add 0.25 mL of 88% formic acid to a 250-mL mixing cylinder containing ~100 mL HPLC grade water. Bring to 250-mL final volume with HPLC grade water. Invert several times to mix. *Prepare weekly.*

4.1.2 *0.088% formic acid in deionized water:* Add 0.25 mL of 88% formic acid to a 250-mL mixing cylinder containing ~100 mL deionized water. Bring to 250-mL final volume with deionized water. Invert several times to mix. *Prepare weekly.*

4.1.3 *Methanol:0.088% formic acid (50:50, v/v):* Add equal amounts of HPLC methanol and 0.088% formic acid in HPLC grade water in an appropriate container. Mix well. *Prepare weekly.*

4.1.4 *5% methanol in DI water:* Add 15 mL methanol to a 500-mL mixing cylinder. Bring to 300-mL final volume with DI water. Invert several times to mix. *Prepare weekly.*

4.1.5 *Ammonium hydroxide:5% methanol in DI water (2:98, v/v):* Add 2.0 mL ammonium hydroxide (~30%) to a 100-mL mixing cylinder. Bring to 100-mL final volume with 5% methanol in DI water. Invert several times to mix. *Prepare weekly.*

4.1.6 *Acetic acid:5% methanol in DI water (2:98, v/v):* Add 2.0 mL acetic acid (glacial) to a 100-mL mixing cylinder. Bring to 100-mL final volume with 5% methanol in DI water. Invert several times to mix. *Prepare weekly.*

4.1.7 *HPLC mobile phase:*

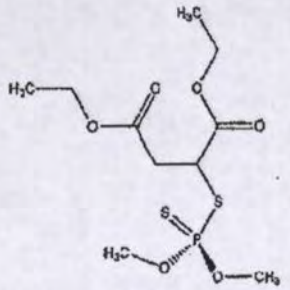
0.1% formic acid in HPLC water: To a 1-liter graduated cylinder, add 1.0 mL of 98% formic acid using a 1.0-mL graduated pipet. Bring to 1-liter final volume with HPLC grade water. Transfer entire solution to the HPLC solvent reservoir and once transferred, mix thoroughly.

5 REFERENCE STANDARDS

5.1 Malathion

Common Name: Malathion
Chemical Names:
CAS: O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate
IUPAC: diethyl (dimethoxythiophosphorylthio)succinate

Structural Formula:



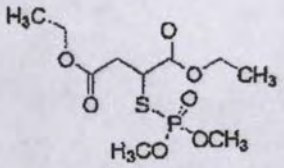
Malathion

CAS No.:	121-75-5
Molecular weight:	330.4
Source:	Cheminova A/S
Storage:	Freezer (typically -8 to -22 °C)

5.2 Malaoxon

Common Name:	Malaoxon
Chemical Names:	
CAS:	Butanedioic acid, [(dimethoxyphosphinyl)-thio]-, diethylester]
IUPAC:	2-(dimethoxyphosphorylthio) butanedioic acid diethyl ester

Structural Formula:



Malaoxon

CAS No.:	1634-78-2
Molecular weight:	314.3
Source:	Cheminova A/S
Storage:	Freezer (typically -8 to -22 °C)

6 STANDARD PREPARATION

All standard solutions prepared in this section are stored refrigerated (1 to 8 °C) in amber bottles, when not in use. Typically the following standard concentrations are prepared:

6.1 Stock Standard Solutions

Twenty-five (25.0) mg (corrected for purity) of malathion and malaoxon are accurately weighed and quantitatively transferred to separate 25-mL volumetric flasks and brought to volume with HPLC acetonitrile. The resulting stock solution concentrations are 1000 µg/mL.

Because it is difficult to weigh small amounts (generally ≤25 mg) of both liquid and solid analytical standards to specific predetermined values, they may be weighed to ±10% of the target value. When calculated, the actual concentration of the stock solution produced is expressed to three significant figures. Appropriate adjustments in the preparation of subsequent dilutions can be made in order to produce concentrations that are more manageable to work with.

6.2 Intermediate/Fortification Standard Solutions

Prepared as individual solutions:

100 µg/mL: Transfer 2.5 mL of the targeted 1000-µg/mL stock standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 12 months.*

10 µg/mL: Transfer 2.5 mL of the targeted 100-µg/mL standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 6 months.*

1.0 µg/mL: Transfer 2.5 mL of the targeted 10-µg/mL standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 3 months.*

Prepared as mixtures:

100 µg/mL: Transfer 2.5 mL of each targeted 1000-µg/mL stock standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 12 months.*

10 µg/mL: Transfer 2.5 mL of a 100-µg/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 6 months.*

1.0 µg/mL: Transfer 2.5 mL of a 10-µg/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare every 3 months.*

0.10 µg/mL: Transfer 2.5 mL of a 1.0-µg/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC acetonitrile. Mix well. *Prepare monthly.*

6.3 HPLC Calibration Standard Solutions

Prepare these mixtures monthly.

2.5 ng/mL: Transfer 625 µL of the 0.10-µg/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC methanol:0.088% formic acid (50:50, v/v). Mix well.

0.50 ng/mL: Transfer 125 µL of the 0.10-µg/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC methanol:0.088% formic acid (50:50, v/v). Mix well.

0.25 ng/mL: Transfer 125 µL of the 0.10-µg/mL mixed standard solution to a 50-mL volumetric flask. Bring to volume in HPLC methanol:0.088% formic acid (50:50, v/v). Mix well.

0.10 ng/mL: Transfer 1.0 mL of the 2.5-ng/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC methanol:0.088% formic acid (50:50, v/v). Mix well.

0.05 ng/mL: Transfer 500 µL of the 2.5-ng/mL mixed standard solution to a 25-mL volumetric flask. Bring to volume in HPLC methanol:0.088% formic acid (50:50, v/v). Mix well.

7 SAMPLE FORTIFICATION

1. Weigh 10.0 g of homogenized soil sample into a 250-mL HDPE centrifuge bottle.
2. Fortify the sample with the appropriate amount of standard solution. Disperse solution over as much of the sample as possible. Use a volume of ≤1.0 mL.
3. Proceed with Step 8.2.

8 SAMPLE EXTRACTION

1. Weigh 10.0 g of homogenized soil sample into a 250-mL HDPE centrifuge bottle. As applicable, fortify appropriate samples at this time.
2. Add 100 mL of acetonitrile. Cap and shake on a platform shaker at medium speed (~180 excursions per minute) for 30 minutes.
3. Centrifuge mixture at ~3000 rpm for ~10 minutes.
4. Decant supernatant (extract) into a 500-mL glass graduated mixing cylinder.
5. Repeat Steps 8.2 and 8.3 two additional times, decanting all extracts into the same cylinder from Step 8.4.
6. Bring the combined extracts to a final volume of 500 mL with acetonitrile. Mix well.

7. Transfer ~30 mL to a 50-mL graduated polypropylene centrifuge tube and cap. Mix well. Centrifuge, if necessary, at ~3000 rpm for ~10 minutes. Remove aliquots for analysis as specified in Step 8.8, and store remainder at 1 to 8 °C as a retain extract of the sample if needed for reanalysis.

8. Transfer 1.0 mL of the extract from Step 8.7 to a 15-mL polypropylene centrifuge tube containing 9.0 mL of 0.088% formic acid in DI water. Cap and vortex-mix. Proceed to Section 9.

9 OASIS® HLB SPE CARTRIDGE CLEANUP

Procedure:

1. Set up the Visiprep system and support apparatus and proceed with Oasis® HLB SPE cleanup. In general, set vacuum to produce a flow rate of approximately 2 mL/minute (not continuous flow) for all elutions.

2. Condition an Oasis® HLB cartridge (size 3 cc, 60 mg) by passing 2 mL methanol followed by 2 mL deionized water, through the cartridge. Do not allow the cartridge to go dry in between any of the conditioning steps or between conditioning and sample introduction. (Stop elution when each conditioning solvent reaches top of frit.) Discard all eluates.

3. Pass the sample extract (10 mL) from Step 8.8 through the SPE cartridge. Stop elution when the solvent reaches the top of the frit. Discard the eluate. Residues of malathion and malaoxon are retained on the cartridge.

4. Add 1 mL of 5% methanol in DI water to the empty centrifuge tube that contained the sample extract. Shake to rinse the tube and pass the rinse through the cartridge. Stop elution when solvent reaches top of frit. Discard eluate.

5. Wash the sample laden cartridge with 1 mL of ammonium hydroxide:5% methanol in DI water (2:98, v/v). Stop elution when solvent reaches top of frit. Discard eluate.

6. Wash the sample laden cartridge with 1 mL of acetic acid:5% methanol in DI water (2:98, v/v). Stop elution when solvent reaches top of frit. Discard eluate.

7. Place a 15-mL polypropylene centrifuge tube, calibrated at 1.0 mL and 2.0 mL, under the SPE cartridge.

8. Elute the analytes with 2.0 mL of HPLC methanol into the calibrated polypropylene centrifuge tube. Allow the cartridge to go dry under vacuum.

9. Using an N-Evap with the water bath set @ 35 °C; concentrate the eluate to the 1.0 mL calibrated mark.

10. Bring to final volume of 2.0 mL with 0.088% formic acid in HPLC water. Sonicate and vortex to mix well. Final sample concentration is 1 mL = 0.01 g sample. Submit to LC/MS/MS analysis.

10 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

Note: The column and conditions stated in the method have been satisfactory for the matrices being analyzed. The specific column packing, mobile phase, column temperature and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

11.1 Operating Conditions

Instrument: Applied Biosystems/Sciex API 4000 LC/MS/MS System with ACQUITY UPLC System including Sample Organizer with Applied Biosystems/MDS Sciex Analyst Software for data collection and system control (version 1.5)

HPLC column: 100 mm x 2.0 mm i.d. Phenomenex Luna C18(2)-HST, 2.5µ particle size

Mobile phase: *Fisher HPLC water, EMD formic acid, Fisher HPLC acetonitrile*

Component A: 0.1% formic acid in HPLC water

Component B: 100% HPLC acetonitrile

Gradient:

Time (min.)	% A	% B
0.0-0.5	75	25
5.0-7.0	5	95
7.1-10	75	25

Flow rate: 0.2 mL/min.

Interface probe: TIS

Ionization mode: Positive (+)

Acquisition mode: MRM

Source temperature: 350 °C

Curtain gas: Nitrogen @ setting of "10"

Collision gas: Nitrogen @ setting of "6"

Injection volume: 10 µL

Column temperature: 40 °C

Autosampler tray temperature: ambient

Transitions monitored:	Ion, *m/z*		Time, *ms*	CE, *v*	
	Q1	Q3			
Malathion:	331	285	150	11	(quantitation)
	331	127	150	19	(alternate)
	331	99	150	19	(alternate)
Malaoxon:	315	127	150	19	(quantitation)
	315	143	150	17	(alternate)
	315	99	150	17	(alternate)

Retention times: Malathion: ~ 5.75 minutes
Malaoxon: ~ 4.30 minutes

10.2 Sample Analysis

Prepare a five-point standard curve by injecting constant volumes of standard solutions. Use constant volume injections for sample extracts as well. For sample responses greater than those produced by the highest concentration of standard curve, dilution and reinjection are required. Inject a curve check standard every 3-4 sample injections.

11 CALCULATIONS

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. The regression functions are used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak response)

and to determine concentrations of the analyte found during sample analysis from the calculated best fit line. **Weighting (1/x) is used.**

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
m	=	slope
x	=	ng/mL found for peak of interest
b	=	y-intercept

The calculations for ppm found and percent recovery (for fortified samples) are:

1. The amount of analyte (in ppm) found in the sample is calculated according to the following equation:

$$ppm = ng/mL\ found \times \frac{final\ vol.(mL)}{sample\ wt.(g)} \times \frac{ext.\ solv.(mL)}{aliq.(mL)} \times \frac{1\ \mu g}{1000\ ng} \times HPLC\ dil.\ factor$$

where:

ng/mL found	=	ng/mL of analyte found from standard curve
final vol. (mL)	=	volume of final extract submitted to instrumentation (typically 2.0 mL)
sample wt. (g)	=	gram weight of sample extracted (typically 10.0 g)
ext. solv. (mL)	=	volume of extraction solvent (typically 500 mL)
aliq. (mL)	=	volume of extract taken through Oasis® HLB SPE cleanup (typically 1.0 mL)
1 µg/1000 ng	=	conversion factor
HPLC dil. factor	=	dilution of sample extract required to produce analyte responses bracketed by standards

2. The percent recovery for fortified control samples is calculated as follows:

$$\%\ Rec. = \frac{ppm\ found\ in\ fortified\ control\ (spike) - ppm\ found\ in\ control}{fortification\ level\ (ppm)\ added} \times 100$$

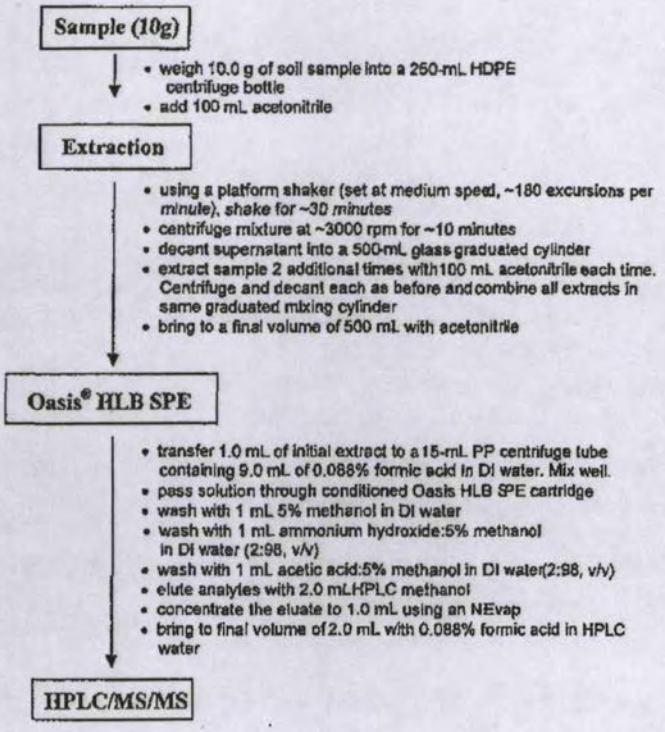
12 REFERENCES

1. Rice, F., Jacobson, B., Lochhaas, C., "Terrestrial Field Dissipation for Malathion in Cotton (California)," Analytical Bio-Chemistry Laboratories Inc., Report No. 38003, Unpublished Report, CHA Doc. No.: 43 FYF, MRID No. 41727701, dated December 3, 1990.

2. Morse Laboratories, "Determination of Malathion and its Metabolites Malaoxon, Desmethyl Malathion, Malathion Monocarboxylic Acid and Malathion Dicarboxylic Acid in Crops (Raw Agricultural and Processed Commodities)," Analytical Method #Meth-198, Original, dated December 16, 2009.

Method author: Kevin Clark

ANALYSIS FLOWCHART



Quality Control for Oasis® HLB SPE Cartridges:

1. Transfer 25 µL of a 0.10-µg/mL malathion and malaoxon mixed standard solution in acetonitrile to a 15-mL PP centrifuge tube containing 1.0 mL of acetonitrile, and 9.0 mL of 0.088% formic acid in DI water. Mix well.
2. Follow Steps 9.1 through 9.6 of the procedure.
3. Place a 15-mL polypropylene centrifuge tube, calibrated at 5.0 mL, under the SPE cartridge.
4. Elute the analytes with 2.0 mL of HPLC methanol into the calibrated polypropylene centrifuge tube. Allow the cartridge to go dry under vacuum.
5. Add 0.5 mL HPLC methanol. Bring to final volume of 5.0 mL with 0.088% formic acid in HPLC water. Mix well.
6. Resulting analyte concentration is 1 mL = 0.50 ng.
7. Submit to LC/MS/MS analysis.