ANALYTICAL METHOD FOR THE DETERMINATION OF OXAMYL AND ITS OXIME METABOLITE IN SOIL USING LC/MS ANALYSIS

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REASON FOR REVISION NO. 1

This revision was created mainly to correct typographical errors entered in the method validation summary data sheets presented in Appendix 2. In addition, the list of related documents (page 9) and references have been updated, and the location of the study raw data within DuPont archives has been clarified. A statement to clarify the definition of a sample recovery outlier, according to the Q-test, has also been added. The analyte peak areas have been added to example chromatograms displayed in figures.

1.0 ABSTRACT

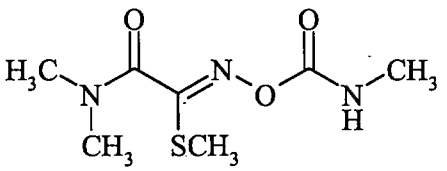
An analytical method has been developed and is described for the determination of trace levels of methyl 2-(dimethylamino)-*N*-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate (hereafter referred to as oxamyl) and methyl 2-(dimethylamino)-*N*-hydroxy-2-oxoethanimidothioate (hereafter referred to as oxime) in soil using LC/MS analysis. Oxamyl and its oxime metabolite are extracted from soil aliquots (approximately 13 g fresh weight) and into organic solvents using the Dionex Accelerated Solvent Extraction ASE® 200 or ASE. Aliquots of the soil extracts are concentrated and diluted for analysis by LC/MS. The instrumental method is liquid chromatographic gradient elution interfaced to a Hewlett-Packard Mass Selective Detector (MSD) using Selected-Ion-Monitoring (SIM). Mode of analysis is Atmospheric Pressure Ionization-electrospray (API-ES) with positive ion mass spectrometric detection of ions having mass/charge (m/z) ratios of 237 ($M+NH_4^+$) for oxamyl and 163 (MH^+) for its oxime metabolite.

2.0 INTRODUCTION

Oxamyl is the active ingredient (a.i.) in the insecticide/nematicide Vydate® L used to control insects, mites, and nematodes in various crops while oxamyl oxime (oxime) is its primary environmental metabolite. This analytical method was developed in order to facilitate the determination of trace levels of oxamyl and/or its oxime metabolite in field treated soil samples in support of the DuPont study AMR 4318-97, "A Small-Scale Prospective Groundwater Monitoring Study for Oxamyl" (Reference 1).

Structures

Oxamyl (DPX-D1410)

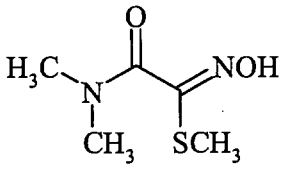


Monoisotopic Mass 219.07

CAS name: Methyl 2-(dimethylamino)-*N*-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate

CAS Number 23135-22-0

Oxime metabolite of Oxamyl (IN-A2213)



Monoisotopic Mass 162.05

Methyl 2-(dimethylamino)-*N*-hydroxy-2-oxoethanimidothioate

CAS Number 66344-33-0

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

HP 1100 Series LC/MSD System – Atmospheric Pressure Ionization Mass Spectrometry (API-MS), HED Mass Analyzer and single-stage quadrupole mass

spectrometer with electrospray source/interface, (Hewlett Packard, Valley Forge, PA).

HPLC Column – Hypersil ODS (2.1 mm x 100 mm, 3-µm), Part # 799160D-352, (Hewlett Packard, Valley Forge, PA).

LC Vials – 2 mL amber LC vials with T/S/T Sepia septum, Part # 5182-0556, (Hewlett Packard, Valley Forge, PA).

Data Acquisition – HP Chemstation, operating under Windows NT software, Version 4, Copyright© 1985-1995, Microsoft Corporation.

Data Analysis, Microsoft Excel for Windows 95, Version 7.0 Copyright© 1985-1995, Microsoft Corporation.

Syringe Filters - Acrodisc®13 CR PTFE, 0.2 µm/13 mm, part number 4423; (VWR Scientific Co.).

Syringes - 3-cc plastic disposable syringe (Beckton Dickinson & Co., Franklin Lakes, NJ).

Balances - Mettler AE160 analytical balance (Mettler Instrument Corp., Hightstown, NJ).

Sartorius Electronic Toploader Model 1400 MP7-2, Part # 01 633 1, (Fisher Scientific, Pittsburgh, PA).

Pipettes - Biohit Proline Electronic pipettors, Variable Volume, (VWR Scientific Co.).

Centrifuge Tube - Kimax, conical, graduated, 13-mL heavy-walled centrifuge tube with stopper, Kimax# 45176 (VWR Scientific Co.).

ASE® 200 - Dionex Accelerated Solvent Extraction, Dionex Corporation (Sunnyvale, CA).

ASE® Filter Paper - 1.91 cm diameter, Grade D 28 Dionex filter paper Part # 049458 Dionex Corporation (Sunnyvale, CA).

ASE® Extraction Cell - 22 mL ASE extraction cell, Dionex Part # 49561, Dionex Corporation (Sunnyvale, CA).

ASE® Collection Vial - 60 mL ASE collection vial, Dionex Part # 48784, Dionex Corporation (Sunnyvale, CA).

Evaporator - N-Evap® Model 111 laboratory sample evaporator/nitrogen manifold (Organomation Associates, South Berlin, MA). Unit is attached to a filtered nitrogen source.

Millex-FG50 filter Hose Barb, 0.2 µm filter unit, 50 mm, Part # SLFG05010 (Millipore Corp., Inc., Bedford, MA).

Mortar and Pestle - Glass, 2 oz., Part # 50415-020, (VWR Scientific Co.).

Ultrasonic Bath - Branson Model 3210 ultrasonic bath (VWR Scientific Co.).

Mixer - Lab-Line Super-mixer, Part # 8294-F10, (Arthur H. Thomas Co., Philadelphia, PA.)

Graduated Cylinders - Pyrex Metric Scale Graduated Glass Cylinder, 50-100 mL, (VWR Scientific Co.).

3.2 Reagents and Standards

Water - Deionized water passed through a Milli-Q® UV Plus water purification system #ZD60 115 UV (Millipore Corp., Inc.).

Methanol (MeOH) - EM Omni Solv®, HPLC-grade methanol, #MX0488-1 (EM Science).

Acetonitrile (ACN) - EM Omni Solv®, HPLC-grade acetonitrile, #AX0142-1 (EM Science).

Sand - Silica Sand, #SX0070-3 (EM Science).

Silica gel - Silica gel 60, #9385-3 (EM Science).

Formic Acid - SIGMA formic acid, # F-0507 (Sigma Scientific).

Ammonium formate - ammonium formate, #AX1300-3 (EM Science).

Reference substance used for HPLC/MS analysis

Oxamyl analytical standard grade, Lot # IN D1410-376, 100.0 % pure (DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Co., Wilmington, DE).

Oxime analytical standard grade, Lot # A2213-10, 99.9% pure (DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Co., Wilmington, DE).

3.3 Safety and Health

Warning: Oxamyl has been classified as a poison. Each analyst must be acquainted with the potential hazards of all the products, reagents, and solvents used in this method before commencing laboratory work. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 Principle of the Analytical Method

A flow diagram of the analytical method from extraction to analysis is shown below.

Method Flow Diagram

Measure ≈ 13 g field moist soil aliquot

↓

Combine and mix with Silica Gel ≈ 2:1 wt:wt

↓

Load soil: silica gel mix into the ASE extraction cell (fill remaining cell void with sand); extract soil with 0.01 % formic acid in 80:20, acetonitrile:methanol, v:v

↓

Measure **Total Extract Volume** collected (~30-35 mL), thoroughly mix the extract and aliquot an **Initial Extract Volume** of 5 mL into a 13 mL, glass centrifuge tube. Add 1 mL of 0.01% formic acid in water and concentrate this aliquot sample under nitrogen to 1.5 mL. Dilute this concentrate to the **Final Extract Volume** of 10 mL using 0.01% formic acid in water, sonicate and shake sample. Further dilute this sample if necessary for analysis purposes using 0.01% formic acid in water to approximate the residues expected in the soil (**Dilution Factor**).

↓

Filter through Acrodisc® 13 PTFE filter

↓

LC/MS – HP-MSD Selected ion monitoring of the ammonium (NH_4^+) adduct ion of oxamyl (237) and the protonated oxime ion (163) using (ES+) electrospray ionisation

4.2 Analytical Procedure

4.2.1 Glassware & Equipment Cleaning Procedures

The effectiveness of any cleaning procedure used should be demonstrated through preparation and the analysis of reagent blanks. In general, all reusable glass and plasticware should be washed in hot tap water with laboratory grade, non-phosphate detergent, rinsed several times with acetone, rinsed several times with distilled water, and allowed to fully dry before use. Care should be taken when working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 Preparation & Stability of Reagent Solutions

0.1% Formic acid in water - Measure 1 L of water. Remove to waste by pipet 1 mL of the water and replace with 1 mL of Formic acid by pipet. Replace every six months or sooner if solution appears turbid or cloudy.

0.01% Formic acid in water – Combine 100 mL of the 0.1% Formic acid solution into 1-L graduated cylinder or volumetric flask and bring to 1.0 L volume with Milli-Q® water. Replace every six months or sooner if solution appears turbid or cloudy.

0.01% Formic acid ASE solution - combine and mix 800 mL acetonitrile with 200 mL of methanol (80:20, v:v). Pipet 0.1 mL of Formic acid into the

acetonitrile:methanol container. Replace every six months or sooner if solution appears turbid or cloudy.

0.1 M Ammonium Formate solution - combine and mix 6.3 g Ammonium Formate into 1 L Milli-Q® water. Replace every six months or sooner if solution appears turbid or cloudy.

0.1 M Formic Acid solution - combine and mix 4.6 g Formic Acid into 1 L Milli-Q® water. Replace every six months or sooner if solution appears turbid or cloudy.

LC Eluents

Eluent A: 1 mM Ammonium Formate in 99:1 Milli-Q® water:methanol v:v. Combine and mix 10 mL of 0.1 M Ammonium Formate solution into 990 mL of 99:1, Milli-Q® water:methanol, v:v. Replace every six months or sooner if solution appears turbid or cloudy.

Eluent B: 99:1 methanol:Milli-Q® water v:v. Replace every six months or sooner if solution appears turbid or cloudy.

4.2.3 *Stock Standard Preparation and Stability*

Standards

Use Class-A volumetric flasks when preparing standard solutions. Where volumetric pipettors are used, they also should be Class A. The absolute volumes of all stock standards, intermediate solutions, and fortification solutions may be varied by the analyst as long as the correct proportions of solute to solvent are maintained and recorded.

The shelf life for the analytical purposes of the stock standards, intermediate stock solutions, and fortification solutions used in this method is estimated to be 2 months. It should be noted that these solutions, when prepared and stored under the conditions described below, showed no significant signs of degradation through hydrolysis, volatilization, or photodecomposition during the 2 month shelf life period.

Stock Standard Solution

Stock standard solutions were prepared by accurately weighing approximately 100 mg of oxamyl into a 100-mL volumetric flask using an analytical balance. The standard was dissolved in approximately 100 mL of acetonitrile in a volumetric flask. The oxime stock standard solution was prepared in a similar fashion. These standard solutions should be stored for a maximum of approximately two months when stored at approximately ≤-10°C. The concentration of each of these solutions in acetonitrile was approximately 1000-µg/mL oxamyl or oxime. The exact concentration of each of the stock standards prepared was recorded, taking the purity of the original material and the actual weight measured into account. The following table illustrates typical stock standard solutions made:

Stock Std	Stock Std I.D. 4318-97-	Stock Std Conc. (mg/mL)	Amount of Oxamyl used to make Std (g)	Final Volume of Oxamyl Std (mL)	Amount of Oxime used to make Std (g)	Final Volume of Oxime Std (mL)	I.D. of Stock Used to make Std	Date Stock Std Made
Oxamyl	84-SS	1.00	0.1000	100.0	-	-	D1410-376	22-Feb-99
Oxime	83-SS	1.00	-	-	0.1000	100.0	A2213-10	22-Feb-99

Intermediate stock standard solutions were made by combining volumes of known stock standard concentrations of oxamyl and oxime and volumetrically diluting the stock standard solutions in acetonitrile as illustrated in the following table of typical intermediate stock standard solutions.

Intermediate Stock Standard	Stock Std I.D. 4318-97-	Stock Solution Conc. (ug/mL)	Date Stock Std Made	Volume of Oxamyl soln. used to make Std (uL)	Volume of Oxime soln. used to make Std (uL)	Final Volume of Oxamyl/Oxime Std (mL)	I.D. of Oxamyl soln. Used to make Std 4318-97-	Date Oxamyl Parent Std Made	I.D. of Oxime soln. Used to make Std 4318-97-	Date Oxime Parent Std Made
Oxamyl/Oxime	112-SS	125.00	25-May-99	1250	1250	10	84-SS	22-Feb-99	83-SS	22-Feb-99

4.2.4 *Fortification Standard Preparation and Stability*

Fortification Standard Solution

Fortification standard solutions were prepared at concentrations of 0.125, 1.25, and 12.5 µg/mL by accurately diluting oxamyl/oxime intermediate stock standard solutions into the appropriate volumes using methanol ("*" in the table below indicates that the fortification solution was raised to volume using 0.01% formic acid in 95:5 Milli-Q® water:methanol, v:v.). Typical dilutions made and concentrations prepared are shown in the following table of fortification solutions. These fortification solutions may be used for at least two months when stored at approximately ≤-10°C. Solutions should be equilibrated to room temperature before aliquots are removed for sample fortifications or chromatographic standard solution preparation.

Fortification Solution	Fortification Solution I.D. 4318-97-	Fortification Solution Conc. (ug/mL)	Date Fort. Soln. Std Made	Volume of Oxamyl/Oxime Stock used to make Soln. (uL)	Final Vol. of Oxamyl/Oxime Fortification Soln. (mL)	I.D. of Parent Stock Soln Used to make Fort. Soln. 4318-97-	Date Parent Stock Soln. Made
Oxamyl/Oxime	113-SS	12.5	25-May-99	1000	10	112-SS	25-May-99
Oxamyl/Oxime	114-SS	1.25	25-May-99	1000	10	113-SS	25-May-99
Oxamyl/Oxime	115-SS	0.125	25-May-99	1000	10	114-SS	25-May-99
Oxamyl/Oxime*	116-SS	0.125	25-May-99	1000	10	114-SS	25-May-99

4.2.5 *Chromatographic Standard Preparation and Stability*

Chromatographic Standard Solutions

The oxamyl/oxime fortification standard solutions were used to prepare the chromatographic standards. Chromatographic standards were prepared over the appropriate range of concentrations as indicated in the table of typical standard solutions below. The corresponding volumes of the fortification standard solutions were transferred into 10-mL volumetric flasks and brought to volume using a 0.01% formic acid in Milli-Q® water:methanol, 95:5, v:v solution.

Oxamyl/Oxime Std I.D. 4318-97-	Final Oxamyl/Oxime Std Conc. (ng/mL)	Volume of Parent Stock Soln used to make Std (uL)	Concentration of Parent Stock Soln used to make Std (ng/mL)	I.D. of Stock Soln used used to make Std 4318-97-	Final Volume of Std (mL)	Standard Preparation Date
54-1	0.125	1000	1.25	118-SS	10	8-Jun-99
54-2	0.50	400	12.5	117-SS	10	8-Jun-99
54-3	1.0	800	12.5	117-SS	10	8-Jun-99
54-4	5.0	400	125	116-SS	10	8-Jun-99
54-5	10	800	125	116-SS	10	8-Jun-99
54-6	20	1600	125	116-SS	10	8-Jun-99

From the date of preparation all calibration standard solutions were stored for a maximum of 2 months in a refrigerator at 2 to 6°C.

Note: after 2 months new standards can be made using the original stock solutions provided the shelf life of the stock solution has not been exceeded. The stability of the calibration standards should be monitored throughout the study by comparing current results for standard solutions with earlier analyses. Any changes/degradation of the calibration standards noticed during the 2 months of the standards shelf life should be noted. If calibration standards are used after two months, they should be

checked against "new" calibration standards to ensure the stability of the "old" standard.

4.2.6 *Source (& Characterization) of Samples*

Soil Samples

This method was developed and validated using soil from an agricultural field in Edgecombe County, North Carolina. Samples of surface soil were collected from the untreated control plot of the study AMR 4318-97, "A Small Scale Prospective Groundwater Monitoring Study for Oxamyl". The soil is sandy (approximately 90% sand w/w), has an organic matter content of about 1% or less, and a soil pH of about 5.8 (Reference 2).

4.2.7 *Storage & Preparation of Samples*

Soil samples are stored frozen at a nominal temperature of ≤-10°C. The procedures for the processing and homogenization of the soil samples are described in the study, "A Small Scale Prospective Groundwater Monitoring Study for Oxamyl", AMR 4318-97 (Reference 3). The soil moisture content was determined by drying a known weight (10 g) of soil sample in a hot air oven at 110°C for up to 48 hours, the sample allowed to cool in a dessicator box, and then re-weighing the soil sample. The weight loss observed over the drying period is expressed as the percentage of moisture content in the soil on a fresh weight basis. The percent moisture content in the soil used for these validation analyses was determined from the average soil moisture content of five (10 g) representative soil aliquots taken from the control plot described in the previous section (4.2.6). The average percent moisture content for the soil used in these validation analyses was determined to be 4.0% with a standard deviation of 0.4% (see Appendix 1).

4.2.8 *Sample Fortification Procedure*

Weigh out 13.0 ± 0.1 g (dry weight ~12.5 g) of control soil to be fortified and extracted into an appropriately labeled mortar identifying the sample used. Add 6.5 ± 0.1 g silica gel 60 to the same mortar (approximately 2:1, g wt: g wt). Gently stir mixture until a uniform mix is observed (avoid excessive grinding of this matrix mixture). Load an ASE® filter paper (1.91 cm) into the bottom of an end-capped 22 mL ASE® cell. Load each sample matrix mix into the open end of an end-capped cell and on top of the filter inserted in the previous step. Gently tap the cell on the bench top during loading. Record all sample identifications and weights to be extracted. The approximately 19.5 total grams of the mix (soil sample: silica gel 60) is not sufficient to completely fill the cell. Fortify directly onto the control matrix samples once they have been loaded into the extraction cell using the spiking solutions described earlier. Allow the cell to remain uncapped for 5-10 minutes to let residual fortification solvents evaporate. Record all volumes and fortification levels. The appropriate volumes of the fortification standard solutions used are added according to the table below for the typical fortification level desired.

Sample I.D.	I.D. of Fortification Soln. Used 4318-97-	Spiking Solution Concentration (ug/mL)	Volume of Spiking Soln. Used (uL)	Nominal Fortification Level (ppb)
A	-	0.000	0	0
B	115-SS	0.125	100	1
C	114-SS	1.250	100	10
D	114-SS	1.250	300	30
E	113-SS	12.50	100	100

Top off each cell with sand to fill the remaining void before capping. This layer of sand helps to prevent clogging of the ASE extraction apparatus. Therefore, it is important to include sand to fill the void created at the top of the cell even if it is the thinnest of sand layers possible.

4.2.9 *Analyte Extraction Procedure*

Oxamyl and its oxime metabolite are extracted from the soil aliquots (approximately 13 g fresh weight) and into organic solvents using the Dionex Accelerated Solvent Extraction ASE® 200 under the following conditions:

Solvent: 0.01% Formic Acid in acetonitrile:methanol 80:20 v:v

Temperature: 50°C

Pressure: 1000 psi

Static Time: 5 minutes

Flush: 100% cell volume solvent flush

Purge: 60 seconds

Transfer the resultant extract from the ASE collection vial to a graduated cylinder and volumetrically measure the total collected extract (**Total Extract Volume**). Transfer approximately 5 mL of the extract to a 13 mL glass centrifuge tube. Record the volume of the aliquot to be concentrated (**Initial Extract Volume**). Add approximately 1 mL of 0.01% Formic acid in Milli-Q® water solution. Concentrate the extract to 1.5 mL on the N-Evaporator with a bath temperature of approximately 40°C and under a gentle stream of nitrogen. **Note**: The flow of nitrogen on the N-evaporator must be regulated to a relatively low rate to minimize aerosol generation and/or evaporative losses of oxamyl. Re-adjust the volume of the extract sample up to approximately 10 mL (**Final Extract Volume**) with a solution of 0.01% formic acid in Milli-Q® water. Further dilution may be necessary to approximate the residues expected in the soil extracts to fall within the linear range of calibration standard concentrations used (**Dilution Factor**). Further dilute this sample if necessary using a solution of 0.01% formic acid in Milli-Q® water.

Mix the extract for 15-30 seconds with the aid of a Vortex Genie™ 2, or a similar mixing device. Place the sample in a sonicator rack and sonicate the sample for 1-2 minutes. Repeat the mixing step and then continue on to the next step.

Filter the diluted extract using a syringe equipped with a 0.2 µm Acrodisc® 13 PTFE (**Do not substitute this filter medium!**) into an appropriately labeled HPLC vial identifying the extract sample.

4.3 Instrumentation

4.3.1 Descriptions and Operating Conditions

Liquid Chromatography

The LC system components were described in the Equipment section of this report. Conditions used for this method are summarized in the following table. The LC conditions may be changed to optimize instrument performance if sensitivity and selectivity requirements are maintained. Note that the retention times may vary slightly on a day to day basis, depending on the batch of mobile phase, etc. Conditions for method validation are summarized in the following table.

LC Conditions:

System		Hewlett-Packard 1100 LC	
Column		Hypersil ODS, 2.1 mm x 10 cm	
Column Temperature		30 C	
Injection Volume		20 uL	
Total Run Time		17.1 min	
Mobile Phase		A) 1mM formate in 99:1 milli-Q water:methanol, v:v	
		B) 99:1 methanol:milli-Q water, v:v	
Gradient:			
Time (min)	Eluent A (%)	Eluent B (%)	Flow (mL/min)
0.00	100	0	0.3
13.00	74	26	0.3
13.10	10	90	0.3
17.00	10	90	0.3
17.10	100	0	0.3

Mass Spectrometry

The HP 1100 LC/MSD mass spectrometer is a benchtop mass selective detector utilizing electrospray ionization. The instrument is operated in positive ion mode, using selected ion monitoring (SIM) to detect ions of mass/charge ratios of 237 for oxamyl and 163 for the oxime metabolite. These ions were selected from the mass spectrum generated during the method development process with the instrument in full scanning mode (See Figure 1 and Figure 2). The conditions outlined below are representative of those used for the particular instrument upon which this method was developed and evaluated.

System	HP 1100 Series LC/MSD System
Source	Atmospheric Pressure Ionization-Electrospray (API-ES)
Ionization Mode	Positive ES+
MS Mode	Selected-Ion-Monitoring (SIM)
Ions Monitored	237 - Oxamyl M+NH4; 163 - Oxime M+H
Data Acquisition	Windows NT Operating Software
Capillary Voltage	Oxime = 3500; Oxamyl = 1500
Fragmentor Voltage	Oxime = 40; Oxamyl = 20
Gain	2
Gas Temperature	340 C
Drying Gas	10.0 L/min
Nebulizer Pressure	Oxime = 35 psig; Oxamyl = 20 psig

4.3.2 *Calibration Procedures*

External standard calibration techniques were used to quantify the amount of oxamyl and its oxime metabolite recovered from the validation soil samples. Chromatographic standards of oxamyl and oxime should be used to calibrate the instrument before and during sample injections. These calibration standards were used to calculate linear calibration functions (peak response or mass abundance over concentration injected ng/mL). See Figure 3. Analyzed samples having detector responses greater than the highest standard within the linear range should be diluted (Dilution Factor) and reanalyzed to fall within the range of standards used. The range of calibration standards used in the validation analyses was from 0.125 to 20.00 ng/mL. The calibration standard responses bracketed the responses of residues found in each analytical set and included a minimum range of 6 concentrations. The correlation coefficient (R) for each calibration curve was ≥0.98 (RSQ ≥0.96).

4.3.3 *Sample Analysis*

This instrumental method uses reversed phase (Hypersil ODS) liquid chromatographic gradient elution interfaced with API-ES to a Hewlett-Packard Mass Selective Detector (MSD) using Selected-Ion-Monitoring (SIM). Mode of analysis is by positive ion mass spectrometric detection of the oxamyl ammonium adduct ion with mass/charge (m/z) ratio of 237 ($M+NH_4^+$) and the protonated oxime ion with mass/charge (m/z) ratio of 163 (MH^+).

Validation of the method described in this report was determined by the recovery data generated from the extraction and analysis of three distinct sets of a total of 17 control soil samples fortified with oxamyl and oxime at 10.0, 30.0, and 100 ppb. Method validation consisted of 3 sets of samples that were extracted and analyzed on three separate days. Each set of validation samples included a minimum of four fortified control samples and one unfortified control sample. The fortified control samples in each sample set included some control samples fortified at 10.0 ppb, one sample fortified at 30.0 ppb, and one sample fortified at 100 ppb for each analyte. If analysis was delayed, samples were stored refrigerated at approximately 2-6°C until analysis could be completed. Each set of the fortified control samples and unfortified control samples analyzed was bracketed by the analysis of a minimum of five calibration standards of oxamyl and its oxime metabolite that were evenly distributed throughout the analytical set. In order to asses the method most (>80%) of the individual percent

recoveries for fortified samples across all fortification levels (10.0, 30.0, and 100 ppb) should be in the range of 70-120%. Within each analytical data set the average percent recoveries should also be in the 70-120% range with a relative standard deviation ≤20%.

The target limit of detection for oxamyl and its oxime metabolite described in this report was conducted at the fortification concentration of 1.0 ppb. Five unfortified control soil samples were carried through the sample extraction and analysis procedures to establish background response in the vicinity of each analyte. Ten additional control soil samples were fortified with oxamyl and its oxime metabolite at 1.0 ppb and carried through identical extraction and analysis procedures. The signal response in the fortified samples in the vicinity of the analyte response should be ≥3 times the background noise response in 90% of the samples to successfully estimate the LOD. A value for noise was determined in each Control matrix sample chromatogram at the retention time of each analyte. Two parallel lines were drawn (parallel line lengths equal to approximately 1.5 times the analyte signal base peak width) encompassing the maximum to the minimum background noise signals in the vicinity of the retention time of the analyte of interest. The distance between the parallel lines was measured in millimeters. Each validation set consisted of the analysis of at least two control matrix samples from which the average noise was determined at the retention time of each analyte. The height of each analyte peak signal response from baseline in fortified control samples (fortified at 1.0 ppb) was also determined in millimeters. A ratio of peak signal to average noise (s/n) was then calculated.

4.4 Calculations

4.4.1 Methods

The mass abundance or peak area response to the ammonium adduct ion of oxamyl (m/z 237; $[M+NH_4^+]$) and the protonated oxime ion (m/z 163; $[M+H^+]$) from chromatographic standard calibration injections was used to establish a calibration function by linear regression. This calibration function was used to calculate the analyte concentrations injected (in ng/g or ppb). The final soil analyte concentrations recovered through analysis were calculated and reported in parts per billion (or ng/g) on a dry weight basis (g water/g dry weight soil).

Where the concentration of the analyte injected onto the detector is equal to:

$$\textit{Analyte Concentration injected } (ng / mL) = \frac{\textit{Peak Area–intercept}}{\textit{Slope}}$$

and:

$$\text{Amountfound(ppb)} = \text{MSDConc.(ng/mL)} \times \frac{\text{Final Extract Vol.(mL)}}{\text{Initial Extract Vol.(mL)}} \times \frac{\text{Total Extract Volume (mL)}}{\text{Total Sample Dry Wt.(g)}} \times \text{Dilution Factor}$$

Therefore:

$$\%\,Recovery = \frac{Amount\ Found\,(ppb)}{Fortification\ Level\,(ppb)}\ x\,100$$

4.4.2 *Examples*

The following example presents a typical fortified control soil sample that was carried through the calculations used to obtain recoveries for this study (these calculations are similar for calculating the recovery of either oxamyl or oxime):

Sample I.D.: Oxamyl control fortified soil sample (10 ppb); 4318-97-OX-56-10 ppb 3 from Method Validation Set # 2 analyzed on 14-Jun-1999 (see Appendix 2).

$$\text{OxamylConc.found(ng / mL)} = \frac{44355.7000 - (-3379.2198)}{25025.2459} = 1.9075\ \mu g / mL$$

$$Oxamyl\ Found\ (ppb) = 1.9075\ ng / mL\ x \frac{10.0\ mL}{5.0\ mL} x \frac{31.5\ mL}{12.48\ g} x\ 1 = 9.6291\ ppb$$

$$\text{Fortification Level (ppb)} = \frac{0.1\ \text{mL}}{12.48\ \text{g}} \text{x} \frac{1.25\ \mu\text{g}}{\text{mL}} \text{x} \frac{1000\ \text{ng}}{\mu\text{g}} = 10.0160\ \text{ppb Oxamyl}$$

$$Oxamyl\ \%\ Recovery = \frac{9.6291\ ppb}{10.0160\ ppb}\ x\ 100\% = 96\%\ Oxamyl\ Recovery$$

Note: Non rounded values were carried all the way through the calculations until the final percent recovery. The final percent recovery was rounded and reported to the nearest whole number according to the default rounding function contained in the Microsoft® Excel 97 SR-2 program.

5.2 ***Timing***

Validation sets for the analytical method consisted of up to eight (8) samples extracted and analyzed per day. The first five to six hours in the day were set aside for the extraction and work up of the samples. LC/MS analysis required approximately 20 minutes per sample (including re-equilibration time between injections). These analyses were run unattended overnight.

5.3 ***Special Precautions***

In order to reduce the probability and frequency of the ASE clogging during the extraction process, it is recommended that a soil:silica gel mixture (2:1, w:w) is used. It is also recommended that in order to achieve a uniform mix that you gently stir the gel mixture with your pestle. Do not attempt to grind this mixture excessively with the pestle.

During the sample concentration step (to 1.5 mL) of the extraction procedure (Section 4.2.9), the N-Evaporator bath temperature (approximately 40°C) and the flow of nitrogen must be regulated at a relatively low rate in order to minimize aerosol generation and/or evaporative losses.

Filter the diluted extract using a syringe equipped with a 0.2 µm Acrodisc®13 PTFE filter and into an appropriately labeled HPLC vial identifying the extract sample. Do not substitute this filter medium unless the substitute filter medium performance is verified. Some filter medium has been associated with loss of recoveries during the method development process.

Generally, carbamate insecticides can hydrolyze under mildly alkaline conditions at room temperature and can be susceptible to volatilization and photodecomposition. Store all extracts, standards, and fortification solutions under the conditions described

in this report. Avoid leaving any of the above solutions out at ambient temperatures for excessive periods of time and store properly when not in use.

5.4 Method Ruggedness

5.4.1 Stability

Validation of the method consisted of a total of 17 samples analyzed in 3 separate sets performed over three days. The recovery results of these analyses indicated that the method was stable over the three-day period.

5.4.2 Specificity/Potential Interference

Due to the mass selective nature of the detection system for this method, observed interference in the vicinity of the retention times for the analytes of interest was less than the target LOD of 1.0 ppb. The soil matrix components do not co-elute or otherwise interfere with the ionization of the analytes of interest.

6.0 CONCLUSIONS

The recovery data from the ASE extraction and LC/MS analysis of fortified control soil samples demonstrated that the method is efficient in the determination of trace levels of both oxamyl and its oxime metabolite in soil. Recovery data supported the target LOD (1 ppb) and LOQ (10 ppb).

Analysis by LC/MS of these control sample extracts indicates that the soil matrix components do not co-elute or otherwise interfere with the ionization of the analytes of interest and that the method is essentially free of signal interference at the retention times of the analytes of interest.

The method developed and validated as described in this report generated percent recoveries of oxamyl and its oxime metabolite that are acceptable for this analytical method to be used to support registration. The study meets U.S. EPA, Subdivision N, 166, U.S. EPA OPPTS Series 850.7100 Guidelines (April 96 Draft), and EEC Directive 91/414EEC: Annex II 4.2.3 criteria.