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

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1.0 ABSTRACT

Oxamyl is the active ingredient inVydate®, an insecticide/nematicide used to control insects, mites, and nematodes in various row, fruit, and vegetable crops. The major metabolite of oxamyl is its oxime metabolite. An analytical method that can detect, quantify, and confirm the presence or absence of these two analytes in water samples is necessary to support registration of oxamyl as an insecticide.

The method described within using liquid chromatography / mass spectrometry / mass spectrometry (LC/MS/MS) analysis was initially written as Centre Analytical Laboratories Inc. (CAL) "Method of Analysis for the Determination of Oxamyl and its Metabolite Oxime in Water Using LC/MS/MS" issued on August 11, 1998. This method has been used to analyze samples generated in two prospective ground water monitoring studies. Validation data and chromatograms generated during these analyses are incorporated.

This method detects, quantitates, and confirms the presence or absence of oxamyl and its oxime metabolite in water. These compounds are analyzed from a 10 mL aliquot of a water sample, following the acidification of the aliquot with an aqueous solution of formic acid. No other concentration or clean-up steps are required. If necessary, the samples are also filtered. Analysis is performed by LC/MS/MS using atmospheric pressure ionization (API) using the multiple reaction monitoring (MRM) mode.

2.0 INTRODUCTION

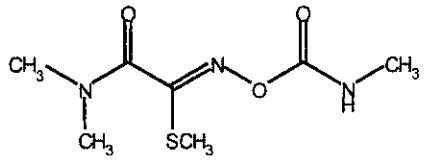
Oxamyl is the active ingredient in Vydate®, an insecticide/nematicide used to control insects, mites, and nematodes in various row, fruit, and vegetable crops. The major environmental metabolite is the oxime metabolite, referred to hereafter as "oxime".

This analytical method can detect, quantitate, and confirm the presence or absence of both oxamyl and oxime in water samples to support registration of oxamyl as an insecticide.

This LC/MS/MS method was initially written as Centre Analytical Laboratories Inc. (CAL) "Method of Analysis for the Determination of Oxamyl and its Metabolite Oxime in Water Using LC/MS/MS" issued on August 11, 1998. This method has been used to analyze samples generated in two prospective ground water monitoring studies. Validation data and chromatograms generated during these analyses are incorporated.

The chemical structure and Chemical Abstracts Service (CAS) names and numbers for oxamyl and oxime are listed below:

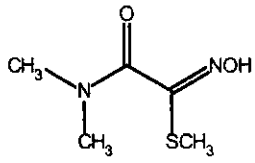
Chemical Structure of Oxamyl (DPX-D1410):



Chemical Name = Methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate

CAS Registry Number = 23135-22-0

Chemical Structure of Oxime Metabolite of Oxamyl (IN-A2213):



Chemical Name = Methyl 2-(dimethylamino)-*N*-hydroxy-2-oxoethanimidothioate

CAS Registry Number = 66344-33-0

Water samples (10 mL aliquots) are prepared for the analysis of oxamyl and its oxime metabolite following the addition of an aqueous formic acid solution. No other concentration or clean up steps are required.

The limit of detection based on the least sensitive analyte is 0.1 ppb with a limit of quantitation defined as 1.0 ppb.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

Acrodisc 13 PTFE (#4423) HPLC sample filters

Standard Lab Equipment (including analytical balances, standard glassware, volumetric flasks, class A volumetric pipets, disposable borosilicate glass pipets etc.)

LC/MS/MS system is described in section 4.3.

3.2 Reagents and Standards

Methanol: JT baker, HPLC grade (VWR Scientific Co., Bridgeport, NJ)

Formic Acid: Formic Acid GR (Em Science, Gibbstown, NJ)

Water: The water used was Type I (LABCONCO WaterPro™ Work station)

Acetonitrile: JT baker, HPLC grade (VWR Scientific Co., Bridgeport, NJ)

Standards: The standards for oxamyl (DPX-D1410, purity 99.6% and purity 100%) and oxime (IN-A2213, purity = purity 99.9%) were obtained from E.I du Pont de Nemours and Company.

3.3 Safety and Health

Warning: Oxamyl is classified as a poison. Each analyst must be acquainted with the potential hazards of the reagents, products 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

This method is based on the method developed at Centre Analytical Laboratories Inc. dated August 11, 1998. It is the method used in two DuPont prospective ground water studies.

A 10 mL aliquot of the sample is acidified with 0.1% formic acid in water. An aliquot is then injected onto a reversed phase liquid chromatography system, using a 150 mm × 4.6 mm Luna phenyl-hexyl column (or equivalent) at a flow rate of 1

mL/min. A water/methanol gradient is used for the separation. The column is interfaced to a triple quadrupole mass spectrometer using a turbo ion spray liquid introduction interface. External standard calibration is used to quantitate the analytes.

4.2 Analytical Procedure

4.2.1 Preparation of Reagent Solutions

(1) 0.1% formic acid is made by adding 1 mL formic acid to 999 mL Type I water.

(2) Mobile Phase A: Type I water (electrical resistivity, minimum of 16.67 MΩ/cm at 25°C) Labconco Waterpro™ Work Station.

(3) Mobile Phase B: Methanol.

4.2.2 Standard Preparation and Stability

Analytical standards are prepared for two purposes. They are used for fortifying untreated samples to determine analytical recovery and also to calibrate the response of the detector used in the analysis.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

4.2.2.1 Stock Standard Preparation and Stability

To prepare stock solutions of 100 µg/mL, weigh out 10 mg of analytical standard (corrected for purity) and bring up to 100 mL with acetonitrile in a 100 mL volumetric flask. Prepare separate solutions for each analyte. These stock solutions are to be stored in a freezer at ≤–10° C and are good for a maximum period of 6 months from the date of preparation.

4.2.2.2 Fortification Standard Preparation and Stability

To prepare a mixed fortification standard of 1.0 µg/mL each of oxamyl and oxime, add 1 mL each of oxamyl and oxime stock solutions to a 100 mL volumetric flask and bring up to volume with 0.1% formic acid in type I water. To make a 0.10 µg/mL mixed fortification standard, 10 mL of the 1.0 µg/mL mixed fortification standard is diluted to 100 mL in a volumetric flask, as described above. One hundred microliters of this mixed fortification standard spiked into 10 mL of matrix is equivalent to 1.0 ppb (1.0 ng/mL) of each analyte.

Store all fortification standard solutions in a refrigerator at 2 to 6°C for a maximum period of 2 months from the date of preparation, after which, new standards should be made using the stock solutions.

4.2.2.3 Chromatographic Standard Preparation and Stability

Five LC/MS/MS calibration standards are prepared in 0.1% formic acid in type I water via dilution of the 0.1 µg/mL (100 ng/mL) fortification solution.

The following is a typical example: additional concentrations may be prepared as needed.

Initial Conc. (ng/mL)*	Volume (mL)	Diluted to (mL)	Final Conc. (ng/mL)*
100	10	100	10
100	5	100	5
10	20	100	2
10	10	100	1
10	5	100	0.5
1	25	100	0.25
1	5	100	0.05

* each of oxamyl and oxime

Store all calibration standard solutions in a refrigerator at 2 to 6 °C for a maximum of 2 months from the date of preparation, after which, new standards should be made using the stock solutions. The stability of the calibration standards should be monitored through out the study by comparing current results for standard solutions with earlier analyses.

Any changes/degradation of the calibration standards should be noted during the 2 months. When new calibration standards are made after two months, they should be checked against the "old" calibration standards to ensure the stability of the "old" standard.

4.2.3 *Source of Samples*

Control ground water used for method development and method validation was provided by the sponsor as a "bulk ground water sample" and was assigned CAL sample ID 987751. The soil-pore water used for method development and method validation were composites of E1 (event 1, pretreatment) lysimeter samples collected for bromide analysis as specified in DuPont protocol AMR 4318-97. These "pooled" soil-pore water samples were identified as CAL sample ID 972965-71 and 972953-58. All samples analyzed using this method, as specified in protocol AMR 4318-97, were provided by DuPont prospective ground water study sites in Edgecombe County, North Carolina and Dorchester County, Maryland, respectively.

4.2.4 *Storage & Preprocessing of Samples*

No sample grinding or homogenization is required for water samples.

Samples should be stored in a freezer at <-10°C upon receipt and allowed to thaw at room temperature prior to taking an aliquot for analysis.

4.2.5 *Sample Fortification Procedure*

Fortified samples are prepared using the 0.10 µg/mL mixed fortification solution. Samples were fortified with each analyte at a concentration of 1.0 ppb by adding 0.1

mL of the 0.10 µg/mL mixed fortification solution to 10 mL of water sample. Samples were fortified with each analyte at a concentration of 10 ppb by adding 1.0 mL of the 0.10 µg/mL fortification standard solution to 10 mL of water sample.

The response for a sample fortified at 1.0 ppb should be equivalent to the 0.5 ng/mL calibration standard response (because the final volume is adjusted to 2-times the initial sample volume as indicated in Section 4.2.6).

4.2.6 ***Analyte Extraction Procedure***

There is no sample extraction procedure. The only sample preparation prior to analysis is acidification and filtration (if necessary).

1. Measure 10 mL of the water sample into disposable 20 mL glass scintillation vial. Fortify the sample if necessary, as described in Section 4.2.5.
2. Adjust the final volume to 20 mL with 0.1% formic acid in water.
3. Transfer approximately 2 mL into HPLC vials for LC/MS/MS analysis (samples containing sediment may need to be filtered using an Acrodisc 13 PTFE filter).

Note: In circumstances where less than 10 mL of the sample is available, any sample volume between 5 and 10 mL could be used, as long as sufficient 0.1% formic acid in water is added to achieve a final volume of 2-times the initial sample volume (i.e, if you use a sample size of 7 mL, adjust the final volume to 14 mL). This final volume should be recorded for each sample.

Sample extracts should be stored in a refrigerator (at approximately 4°C) until the time of analysis. During the method development stage, sample extracts containing both analytes were stable for at least one week when stored refrigerated (at 2 to 6°C).

4.3 ***Instrumentation***

4.3.1 ***Description***

Instrument: PE SCIEX API III Biomolecular Mass Analyzer
SCIEX Turbo Ion Spray Liquid Introduction Interface
Harvard infusion pump

Temperature & Gas Flow Settings:
Interface temperature = 60° C
Curtain gas flow (nitrogen) = ~ 0.9 L/min
Nebulizer gas flow = ~ 1.0 L/min
Curtain gas thickness (CGT) = ~ 300
Turbo Ion spray temperature = 400° C
Auxillary gas flow = ~ 5.0 L/min

Computer: Apple Macintosh Quadra 950
Software: Macintosh system 7.5.3, PE Sciex Tune 2.5

PE Sciex RAD 2.6, Multiview 1.2, MacQuan 1.4

HPLC: Shimadzu SCL-10A system controller
Shimadzu LC-10AD solvent delivery modules
Shimadzu SIL-10A auto injector
Shimadzu CTO-10A column oven

HPLC Column: Phenomenex, Luna Phenyl-Hexyl, 4.6 x 150 mm, 3µm,
OR: Keystone, Betasil C6, 4.6 x 150 mm, 5µm

Inline filter: Rheodyne, 0.5µ pore size × 1.5 mm diameter (optional)

Column Temperature: 35° C

Mobile Phase (A) : Type I water (degassed)

Mobile Phase (B) : Methanol (degassed)

Time	% A	% B
0	80	20
6	50	50
7	30	70
7.5	0	100
8.5	0	100
9	80	20
11	80	20

Flow Rate: 1.0 mL/min

Injected Volume: 25 to 100 µL (the injection volume should be recorded for each sample set).

Note: the injection volume used should achieve a ~ 3:1 signal:noise ratio for the 0.05 ng/mL standard.

The HPLC conditions may be changed in order to optimize instrument performance.

Analyte	Retention Time (min)
Oxamyl	~ 6.8
Oxime	~ 5.0

Note that the retention times may vary on a day to day basis, depending on the batch of mobile phase etc. Drift in retention times, within an analytical run, is acceptable as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

4.3.2 *Operating Conditions*

Oxime and oxamyl are analyzed using two "state files", using two "periods" of data acquisition.

The state file parameters are shown below (period 1 = oxime, period 2 = oxamyl). Note that the State File Parameters are given as an example and may need to be changed in order to optimize instrument performance.

Scan type: MRM
Polarity: Positive
Acquisition mode: Profile

Masses requested:

Analyte	Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
Oxime	163	72	750
Oxamyl	220	72	750

State Table Parameters:

Name	Parmeter	Period 1	Period 2
Discharge Current	DI	50	50
Ion Spray Voltage	ISV	4000	4300
Interface Plate	IN	650	650
Orifice	OR	62	45
Quad 0 Rod Offset	R0	30	30
Quad 1 Mass	M1	1000	1000
Quad 1 Resolution	RE1	126.6	126.6
Quad 1 Delta Mass	DM1	0.140	0.140
Quad 1 Rod Offset	R1	26	27
Lens 7	L7	19	20.5
Quad 2 Rod Offset	R2	14	14
Quad 3 Mass	M3	1000	1000
Quad 3 Resolution	RE3	123.4	123.4
Quad 3 Delta Mass	DM3	0.110	0.110
Q2 Lense	RX	6	6
Quad 3 Rod Offset	R3	6	6
Lens 9	L9	-200	-200
Faraday Plate	FP	-200	-200
Electron Multiplier	MU	-4200	-4200
Count Control	CC	1	1
Collision Gas Valve	CG	Ar	Ar

The mass spectrometer is tuned by infusing a ~ 0.5 µg/mL standard solution of each analyte (at 10 µL/min). The analytes are initially tuned for the parent ion in Q1 and then tuned for the product ion in Q3. The optimized parameters are saved as "state files", to be used during routine analysis.

4.3.3 ***Calibration Procedures***

Inject an aliquot (25 to 100 µL) of each calibration standard in the range of 0.05 ng/mL to 5 ng/mL into the LC/MS/MS. The 0.05 ng/mL calibration solution is included to demonstrate the method detection limit, which has been targeted at 0.1

ppb (this standard may or may not be included in the construction of the calibration curve). The injection volume may be adjusted, as needed, in order to achieve an approximately 3:1 signal:noise ratio for the 0.05 ng/mL calibration solution (the injection volume should be constant throughout a set).

Inject the same aliquot of each sample/fortification/control into the LC/MS/MS. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. If necessary, dilute the samples to give a response within the standard curve range.

A standard should be the first and last injection in a sample set, and standards should be injected at least as frequently as after every three to four treated or control samples.

A linear $y = mx + b$ function is used for quantification. Calibration curves may be generated using the MacQuan software system for each set of analysis. The correlation coefficient (R) for calibration curves generated should be ≥ 0.98 ($R^2 \geq 0.96$). If calibration results fall outside these limits, then appropriate steps should be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

A calibration curve is generated for each analyte by plotting the response of the mass spec detector in peak area versus the concentration of each analyte standard that was injected. A typical calibration curve can be found in Appendix 1.

4.3.4 Sample Analysis

Standards and extracts are analyzed using a reversed phase liquid chromatography analytical column (150 mm x 4.6 mm) interfaced to a triple stage mass spectrometer via atmospheric pressure ionization using a turbo ion spray liquid introduction interface (TIS). The chromatographic separation is performed using a gradient elution. The analysis is performed by positive ion MS/MS using the protonated molecular ion as the precursor. Product ions are formed by collision induced dissociation of the precursor ion in the collision cell of the mass spectrometer. The predominant product ion is then mass analyzed in the third quadrupole filter.

Each set of samples analyzed for investigation purposes should include at least one control sample and two samples fortified with oxamyl and oxime, at known levels and carried through the procedure to verify recovery. The validation results described in this report were conducted at fortification concentrations of 1.0, 3.0, and 10 ppb for each analyte.

If analysis is delayed, samples should be stored refrigerated at approximately 4°C until analysis, and analyzed within a week.

Fortifications that bracket the highest residue found in each treated sample should be included with each sample set or included in a subsequent sample set to establish that the method recoveries of all analytes of interest are at concentrations exceeding those in treated samples.

4.4 *Calculations*

4.4.1 *Methods*

1. The oxamyl and oxime concentrations found in the HPLC injection solution is calculated in the following way (using external standard, regression analysis):

   A standard curve (containing at least 4 concentrations) is constructed using peak areas of known standard injections versus the concentration (ng/mL) of the solutions injected. Linear least squares regression is applied to the curve to generate the standard curve equation: peak area = (slope) (concentration injected, ng/mL) + intercept. The oxamyl or oxime concentration for each injection solution is calculated from the standard curve equation using the chromatographic peak area. (The standard curves are also manually plotted for visual inspection.)

2. Oxamyl and oxime residues are reported in units of parts per billion (ppb, ng/mL). The following equation is used to calculate oxamyl or oxime concentration in the sample.

   oxamyl or oxime concentration (ppb) =

   $$\text{concentration injected (ng/mL)} \times \frac{\text{final volume (mL)}}{\text{initial sample aliquot (mL)}} \times \text{dilution factor (if any)}$$

   where:

initial sample aliquot (mL)	=	10 mL for water analyzed
final volume (mL)	=	20 mL in the case of no dilution
dilution factor (if any)	=	ratio of volume of diluted solution for injection to the volume of injection solution if dilution was not performed

   $$\text{concentration injected (ng/mL)} = \frac{\text{peak area - intercept}}{\text{slope}}$$

4.4.2 *Calculation of Fortification Recovery*

1. Find parts per billion in fortified sample as described above.
2. Use the following equation to calculate recovery:

   $$\% \text{ Recovery} = \frac{\text{oxamyl or oxime concentration (ppb)}}{\text{fortification level (ppb)}} \times 100$$

   where:
   fortification level (ppb) can be calculated as follows:

   fortification level (ppb) =

$$\text{volume fortification standard (mL)} \times \frac{\text{concentration fortification standard } (\mu g / mL)}{\text{sample volume (mL)}} \times \frac{1 \text{ mL}}{1 \text{ g}}$$

3. Report % recovery to the nearest whole number.

Non-rounded values were used for the previous calculations until the calculation of the amount found was calculated and rounded according to Microsoft Excel's default rounding function.

4.4.3 ***Examples***

An example of the previous calculations is presented here using an actual fortified bulk ground water sample from the method validation conducted under DuPont protocol AMR 4318-97. The sample was assigned CAL sample ID 987751 Spk F1 (data set # 092498A, extracted and analyzed on 9/24/98). See Appendix 1:

The ppb found calculated for Oxamyl (oxime is calculated in the same manner):

$$\text{concentration injected (ng/mL)} = \frac{\text{peak area - intercept}}{\text{slope}}$$

$$= \frac{766 - 155.948809}{1185.44473} = \mathbf{0.514618} \text{ ng/mL}$$

$$\text{oxamyl or oxime concentration (ppb)} =$$

$$\text{concentration injected (ng/mL)} \times \frac{\text{final volume (mL)}}{\text{initial sample aliquot (mL)}} \times \text{dilution factor}$$

$$= \frac{0.514618 \times 20.0 \times 1}{10.0} = \mathbf{1.03} \text{ ppb}$$

$$\% \text{ Recovery} = \frac{\text{oxamyl or oxime concentration (ppb)}}{\text{fortification level (ppb)}} \times 100$$

$$= \frac{1.03 \times 100}{1.0} = \mathbf{103\%}$$