Bayer CropScience TD-002-W13-01

1. Principle

The residues of Thiodicarb and its metabolite Methomyl in surface water are determined by solid phase extraction with an Oasis HLB cartridge. The eluent is dried under nitrogen and reconstituted in methanol. The resulting sample is analyzed by LC/MS/MS. Quantification is based on a comparison of peak areas with those of known standards. Two sets of MRM transitions are shown, one for quantitation and the second for confirmatory purposes.

The method limit of quantitation (LOQ) for Thiodicarb and its metabolite Methomyl is 0.1 µg/L in water.

2. Apparatus

Use as a guide; equivalent apparatus may be substituted.

VWR Pyrex® Brand volumetric pipets, glass class A (Assorted Volumes)
Eppendorf Reference and Repeat pipettes and tips
VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)
VWR Pyrex® Brand disposable Pasteur pipets (Cat. No.: 53283-910 & 53283-914)
Kimble 15mL disposable conical-bottom glass centrifuge tubes (Cat. No.: 73790-15)
BD Falcon 50mL conical centrifuge tubes (Cat. No.: 1443222)
National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)
National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)
Supelco® Ascentis® Express C18 10 x 2.1 mm Column (Cat No.:53823-U)
ABSciex API 5500 chromatograph/mass spectrometer (LC-MS/MS) equipped with electrospray ionization (ESI) interface, Shimadzu HPLC pumps and a CTC PAL autosampler, and Analyst 1.6.1 data collection software or higher version, or equivalent
TurboVap evaporator (Zymark Corporation, Model LV)
Centrifuge
SPE Manifold
PTFE Needles for SPE Manifold (Grace, 2107149)
Waters® Oasis HLB 1cc Vac RC Cartridge, 60mg sorbent (Cat. No.: 186000381)
Various general laboratory glassware and utensils

3. Reagents

Use as a guide; equivalents or different manufactures (brands) may be substituted.

Water (HPLC Grade or Millipore)
Methanol (HPLC Grade)
Dichloromethane (HPLC Grade)
L-Cysteine hydrochloride (Fisher BP376)
Ammonium formate (HPLC Grade)
Formic acid (HPLC Grade)
50mg/mL L-Cysteine HCl in Methanol. (example preparation, dissolve 5g of L-Cysteine hydrochloride to 100mL with Methanol)

50µg/mL L-Cysteine HCl in Methanol. (example preparation, dilute 1mL of 50mg/mL L-Cysteine HCl to 1L with Methanol)
Methanol/Dichloromethane solution (3/1). (example preparation, combine 25mL Dichloromethane and 75mL Methanol)
90/10 Water/Methanol with 10mM ammonium formate and 120µL/L formic acid. (example preparation, combine 900mL water and 100mL Methanol. Weight out 6.306mg ammonium formate and add to 90/10 mixture. To that mixture at 120µL formic acid. Mix well.
10/90 Water/Methanol with 10mM ammonium formate and 120µL/L formic acid. (example preparation, combine 100mL water and 900mL Methanol. Weight out 6.306mg ammonium formate and add to 90/10 mixture. To that mixture at 120µL formic acid. Mix well.

4. Preparation of Analytical Standards

NOTE: The following procedure is an example description of how standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a freezer when not in use. Solutions should be allowed to warm to room temperature prior to use.

4.1 Primary Stock Standard Solutions

Prepare individual 100µg/mL stock solutions of Thiodicarb and Methomyl by transferring 0.0100 grams of each analyte to separate 100mL volumetric flasks. Dilute to volume with 50µg/mL L-Cysteine HCl in Methanol and mix well. Store at <-10°C when not in use.

Prepare individual 10µg/mL solutions of Thiodicarb and Methomyl by taking a 2.5mL aliquot of the 100µg/mL stock solutions and diluting to 25mL with 50µg/mL L-Cysteine HCl in Methanol. Store at <-10°C when not in use.

Prepare individual 0.1µg/mL solutions of Thiodicarb and Methomyl by taking a 0.25mL aliquot of the 10µg/mL stock solutions and diluting to 25mL with 50µg/mL L-Cysteine HCl in Methanol. Store at <-10°C when not in use.

Prepare individual 0.01µg/mL solutions of Thiodicarb and Methomyl by taking a 2.5mL aliquot of the 0.1µg/mL stock solutions and diluting to 25mL with 50µg/mL L-Cysteine HCl in Methanol. Store at <-10°C when not in use.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

4.2 Mixed Standard Solutions

Prepare a mixed 10µg/mL solution of Thiodicarb and Methomyl by taking 1mL of each 100µg/mL stock standard solution and diluting to 10mL with 50µg/mL L-Cysteine HCl in Methanol and mix well. Store at <-10°C when not in use.

Prepare a mixed 1µg/mL solution of Thiodicarb and Methomyl by taking 1mL of the 10µg/mL mixed solution and diluting to 10mL with 50µg/mL L-Cysteine HCl in Methanol and mix well. Store at <-10°C when not in use.

Prepare a mixed 0.1µg/mL solution of Thiodicarb and Methomyl by taking 1mL of the 1µg/mL mixed solution and diluting to 10mL with 50µg/mL L-Cysteine HCl in Methanol and mix well. Store at <-10°C when not in use.

Prepare a mixed 0.01µg/mL solution of Thiodicarb and Methomyl by taking 1mL of the 0.1µg/mL mixed solution and diluting to 10mL with 50µg/mL L-Cysteine HCl in Methanol and mix well. Store at <-10°C when not in use.

4.3 Fortification Standard Solutions

The individual 0.1µg/mL and 0.01µg/mL solutions of Thiodicarb and Methomyl prepared in Section 4.1 above may be used as the fortification solutions for the LOQ and 10X fortifications. Due to the rapid degradation of Thiodicarb to Methomyl, fortifications for each analyte must be performed separately.

Further dilutions of this mixed fortification solution may be made as needed.

4.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.1, 0.2, 1, 2, 10, and 20ng/mL of Thiodicarb and Methomyl as described in the following table using the mixed standard solutions and diluting all solutions to 10mL with 50µg/mL L-Cysteine HCl in Methanol.

Concentration of Standard Solution used for dilution (µg/mL)	Aliquot Native mix Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)
0.01	0.1	10	0.1
0.01	0.2	10	0.2
0.1	0.1	10	1
0.1	0.2	10	2
1	0.1	10	10
1	0.2	10	20

Additional calibration solutions may be prepared as required. Store in freezer at <-10°C when not in use. All solutions are stable in the freezer for approximately one week.

5. *Extraction*

NOTE: The analytical targets in this method are subject to rapid degradation and the extraction should be completed in a timely manner. It is recommended that the conditioning of the SPE cartridges in steps 3 to 5 below is performed concurrently with steps 1 to 2 in such a manner that the samples can be loaded onto the conditioned SPE (step 6) as quickly as possible after being taken off the centrifuge.

1. Transfer 10mL of sample water to a 50mL conical centrifuge tube and fortify the recovery samples at the desired fortification level as needed using the individual standard solutions (see Section 4.3 Fortification Standard Solutions). Cap and briefly shake to mix.
2. Centrifuge surface water at ≈12,500g for ≈3 minutes to remove any particulates.
3. Label SPE cartridges, attach PTFE needles, and affix to an SPE manifold.
4. Condition cartridges with ≈2mL of Methanol by gravity flow.
5. Condition cartridges with ≈2mL of Water by gravity flow.
6. Run 5mL of centrifuged sample through cartridge by gravity flow.
7. Dry cartridges by applying vacuum at ≈20" Hg for ≈15 minutes.
8. Discard all flow-through fluids and place labeled 15mL centrifuge tubes under each cartridge.
9. Elute with 2mL Methanol by gravity flow. Dry cartridge by applying vacuum at ≈5" Hg for ≈1 minute.
10. Elute with 1mL Methanol/Dichloromethane solution (3/1). Dry cartridge by applying vacuum at ≈5" Hg for ≈1 minute.
11. Repeat step 10.
12. Dry samples in Turbo-Vap at 45°C, ≈20psi (approximately 30 minutes).
13. Reconstitute samples in 2.5mL L-Cysteine HCl in Methanol and vortex for ≈20 seconds.
14. Transfer samples to LC vials. Cap the vial and the sample is ready for analysis by LC/MS/MS. If the samples will not be analyzed immediately, they should be stored at <-10°C and then be allowed to warm to room temperature immediately before analysis.

6. Analysis

6.1 Sample Analysis

Step 1. Using the recommended procedures listed below; analyze an aliquot of the 0.1, 0.2, 1, 2, 10, and 20ng/mL standard solutions followed by two blank injections of L-Cysteine HCl in Methanol (these are calibration solution analyses). Analyze the entire curve plus blanks 4 times in a row discarding the data from the first three replicates.

Step 2. Analyze an aliquot of each analytical sample from Section 5 Step 14.

Step 3. Again analyze an aliquot of the 0.1, 0.2, 1, 2, 10, and 20ng/mL calibration standard solutions.

6.2 LC/MS/MS Standard Calibration and Residue Calculations

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution interspersed with samples.

The residues of Thiodicarb and its metabolite Methomyl are quantified using external standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the standard peak area versus the standard concentrations in ng/mL using ABSciex Analyst Software (Version 1.6.1), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients M and B, respectively called slope and intercept, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

where: X *is the concentration of the reference standard in ng/mL*
M *is the calibration line slope*
B *is the calibration line intercept*
Y *is the native peak area*

The calibration points are weighted 1/x to provide better fit near the limit of detection.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of Methomyl in the water was calculated using the following equation,

$$Methomyl\ found\ (ppb) = \frac{Y-B}{M} \times D$$

$$Dilution\ Factor\ (D) = \frac{Final\ volume\ (V2)}{Initial\ volume\ (V1)}$$

$V1 = 5mL$

$V2 = 2.5mL$

Analyst software was used to calculate the residues of Methomyl in ppb for each sample

and the percent recovery for the fortified samples. Due to the rapid degradation of Thiodicarb, its recovery must be determined as the total residue of Thiodicarb and Methomyl.

$$Total\ Residue\ found\ (ppb) = (P \times \frac{Y_M - B_M}{M_M} + \frac{Y_T - B_T}{M_T}) \times D$$

$$Parent\ Equivalency\ Factor\ (P) = \frac{Parent\ Molecular\ Weight}{Product\ Molecular\ Weight} = \frac{354}{162}$$

$$Dilution\ Factor\ (D) = \frac{Final\ volume\ (V2)}{Initial\ volume\ (V1)}$$

V1 = 5mL

V2 = 2.5mL

Subscripts M or T indicate the calibration curve from which the standards Y, B, and M are derived

6.3 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation.

$$Recovery\ (\%) = \frac{R}{T} \times 100$$

Where: *R* = *ppb of target analyte found in fortified sample*
T = *theoretical ppb in fortified sample*

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 0.1ng/mL or other appropriate level with fortification solutions. Calculate the final residue R for the fortified control (R) samples.

Appendix 1 Instrument Conditions for Thiodicarb and Its Metabolite Methomyl

Equipment with equivalent or better sensitivity and performance may be substituted.

LC/MS/MS Parameters

NOTE: Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

The following conditions were used on an ABSciex API 5500 LC/MS/MS system.

HPLC Parameters

Pumps Used:	Two Shimadzu LC-20ADXR pumps with a Shimadzu CBM-20A controller	
Autosampler	CTC PAL	
Column Temperature:	45°C	
Injection Volume:	5uL	
Column:	Manufacturer:	Supelco®
	Type:	Ascentis® Express C18
	Particle Size:	2.7µm
	Diameter:	2.1 mm
	Length:	100 mm
Mobile Phase A:	90/10 Water/Methanol with 10mM ammonium formate and 120µL/L formic acid	
Mobile Phase B:	10/90 Water/Methanol with 10mM ammonium formate and 120µL/L formic acid	

HPLC gradient program:

Time (min.)	Module	Flow Rate (mL/min)	A(%)	B(%)
0.0	Pumps	0.50	95	5
0.5	Pumps	0.50	95	5
0.51	Pumps	0.50	30	70
1.5	Pumps	0.50	0	100
2.5	Pumps	0.50	0	100
2.51	Pumps	0.50	95	5
5.0	System Controller	Stop		

Diverter valve program :

Time (min.)	Position
0.0	Waste
0.9	Source
3.0	Waste

Integration :

	Thiodicarb	Methomyl
Smoothing width	3	3
Noise percent	95	95
Peak-splitting factor	1	0
Expected RT	1.5	1.3

Mass Spectrometer Instrument Conditions

Component:	Thiodicarb	Thiodicarb	Methomyl	Methomyl
Retention Time	1.5 minutes	1.5 minutes	1.3 minutes	1.3 minutes
Transition	Quantitation	Confirmation	Quantitation	Confirmation
Parent Ion	355	355	163	163
Product Ion	88	108	88	106
Ionization Mode	ESI	ESI	ESI	ESI
Polarity	+	+	+	+
Dwell Time (ms)	150	150	150	150
Declustering Potential (DP)	56	56	36	36
Entrance Potential (EP)	10	10	10	10
Collision Energy (CE)	15	21	13	13
Collision Cell Exit Potential (CXP)	12	8	12	8
Curtain Gas (CUR)	20	20	20	20
Collision Gas (CAD)	7	7	7	7
Ion Source Gas 1 (GS1)	21	21	21	21
Ion Source Gas 2 (GS2)	5	5	5	5
Source Temp (TEM)	500	500	500	500
Interface Heater (IHE)	On	On	On	On
Ion Transfer Voltage (IS)	5500	5500	5500	5500

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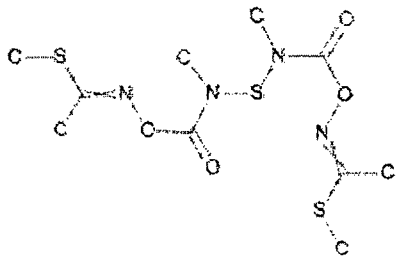
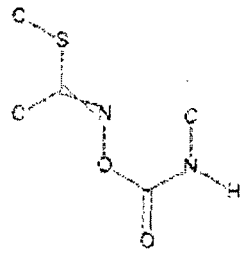
Appendix 2 Structures

Chemical Name: Thiodicarb
(Parent Molecule)



CAS Name: Dimethyl N,N'-
[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]
CAS Number 59669-26-0
Molecular Formula: $C_{10}H_{18}N_4O_4S_3$
Molecular Weight: 354.5

Chemical Name: Methomyl
(Metabolite)



CAS Name: Methyl N-[[(Methylamino)carbonyl]oxy]ethanimidothioate
CAS Number 16752-77-5
Molecular Formula: $C_5H_{10}N_2O_2S$
Molecular Weight: 162.2

APPENDIX 4. Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. LIMS was used to calculate the ppb and percent recovery and the data in Microsoft® Excel spread sheets. The following equations were used for quantitation:

The following equations are used for residue calculations within MassHunter:

a) Calibration curve $y = mx + b$: Solving for x: $x = \frac{y - b}{m}$

Where, m = Slope
b = y intercept
x = Amount found (pg)
y = Peak area

The pg found was calculated by the data system from the linear regression analysis of the data.

b) $\mu L \text{ of sample injected} = \frac{\text{Sample weight (mL)} \times \text{Injection volume } (\mu L)}{\text{Final sample volume (mL)}} \times DF$

c) $\text{Analyte concentration (ppb)} = \frac{\text{pg Found}}{\text{Injection volume } (\mu L)}$

d) $\begin{pmatrix}\text{Total residues of} \\ \text{Thiodicarb} \\ \text{and Methomyl} \\ \text{(ppb)}\end{pmatrix} = \left[(P \times \text{Methomyl} \frac{pg}{\mu L} \text{ Found}) + \text{Thiodicarb} \frac{pg}{\mu L} \text{ Found}\right] \times DF$

$$\text{Parent Equivalency Factor (P)} = \frac{\text{Parent molecular weight}}{\text{Product molecular weight}} = \frac{354}{162}$$

$$\text{Dilution Factor (DF)} = \frac{\text{Final volume (V2)}}{\text{Initial extract volume (V1)}} = \frac{2.5}{5}$$

e) $\text{Percent recovery} = \frac{\text{Total residues in recovery sample} - \text{average residue in the control}}{\text{ppb added}} \times 100$