

FINAL REPORT

TITLE

Independent Laboratory Validation (ILV) of the
BAYER Method TD-002-W13-01: The Determination of Thiodicarb and
its Metabolite Methomyl in Water using LC/MS/MS

TEST GUIDELINES

US EPA Test Guidelines:
OCSPP 850.6100, OPPTS 850.7100 and OCSPP 860.1340(c) (6)

2.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Bayer Analytical Method TD-002-W13-01, "An Analytical Method for the Determination of Residues of Thiodicarb and its Metabolite Methomyl in Water Using LC/MS/MS" performed by ADPEN Laboratories, Inc. The analytical method is presented in Appendix 1.

This study was designed to satisfy harmonized guideline requirements described in US EPA Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Reference 2), OPPTS 850.7100 (Reference 3), and OPPTS (OCSPP) 860.1340(c) (6) (Reference 4). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (Reference 5).

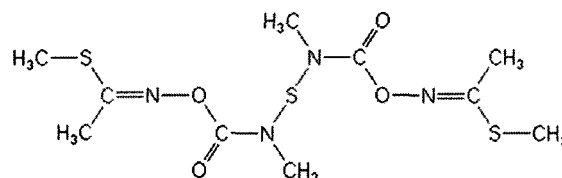
3.0 MATERIALS AND METHODS

3.1 Reference Substances

The following reference substances were obtained from Bayer CropScience:

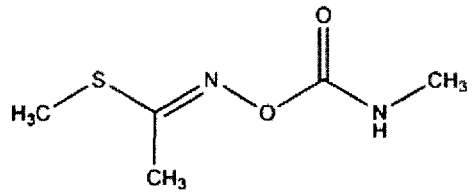
Standard name: Thiodicarb
IUPAC name: dimethyl-*N,N'*-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]
CAS number: 59669-26-0
Empirical formula: C₁₀H₁₈N₄O₄S₃
Molecular Weight: 354.47 g/mol
Standard Number: K-1470
Ref. Substance Lot: 0428200301
GLP purity: 99.8%
Expiration date: 10-Feb-2019
Storage conditions: Refrigerator (E-109)

Molecular structure:



Standard name: Methomyl
IUPAC name: methyl-*N*-[[[(methylamino)carbonyl]oxy]ethanimidothioate]
CAS number: 16752-77-5 (E: 19929-35-9; Z: 19928-37-1)
Empirical formula: C₅H₁₀N₂O₂S
Molecular Weight: 162.21 g/mol
Standard Number: K-2059
Ref. Substance Lot: 1218200306
GLP purity: 99.5%
Expiration date: 11-Feb-2018
Storage conditions: Refrigerator (E-109)

Molecular structure:



3.2 Test System

The test system for this study was an in-house bulk control surface water sample, which was

The sample was logged into the study through the Laboratory Information Management System (LIMS) and assigned a unique laboratory code, which is cross-referenced to the sample number on raw data and detailed residue reports. During the course of this study, the sample was stored in freezer E-16, which operates at a temperature of less than -20°C .

Sample extracts from the analytical set for thiodicarb (WO-13052401) were injected immediately after extraction. Sample extracts from the analytical set for methomyl (WO-13052404) were stored for one day in freezer E-51 while awaiting LC/MS/MS analysis. The temperature for freezer E-51 at this time was approximately -20°C .

3.3 Analytical Procedures

Analytical Method TD-002-W13-01 was independently validated as written. The apparatus and reagents were used as outlined in the analytical method, see Appendix 1, with equivalent apparatus or reagents substituted as necessary.

3.3.1 Fortifications

Untreated control water samples were fortified using the appropriate fortification standard at LOQ (0.1 ppb) and $10\times$ LOQ (1.0 ppb) concentrations as per the method. Fortifications used in this method validation are below.

Matrix	Fortification Volume (mL)	Fortification Concentration (ng/mL)	Sample Wt. (mL)	Final Concentration (ppb)	Replicates
Water	0.1	0.01	10.0	0.1	5
	0.1	0.10	10.0	1.0	5

3.3.2 Extraction Procedure

1. A 10-mL aliquot of water was transferred in to a 50-mL disposable polypropylene centrifuge tube.
2. SPE manifold with PTFE needles was prepared and Waters Oasis HLB 1cc Vac RC 60 mg cartridge was attached.
3. The cartridges were conditioned with 2-mL of methanol (MeOH) followed by of HPLC-grade Water (2 mL). The sample was eluted by gravity.
4. Recovery samples were fortified using individual standard solutions.

5. Samples were capped and briefly shaken to mix and centrifuged at 4000 RPM for 10 minutes.
6. A 5-mL aliquot from Step 5 was loaded on HLB cartridge and was eluted by gravity.
7. Approximately 20" Hg of vacuum was applied for 15 minutes and eluate was collected.
8. A 15-mL centrifuge tube was placed under each cartridge.
9. Sample was eluted by gravity with MeOH (2 mL) and the column was dried by applying approximately 5" Hg vacuum for 1 minute followed by MeOH/DCM solution (3:1, 1 mL v/v) eluting by gravity.
10. Step 9 was repeated.
11. Samples were evaporated to dryness in N-Evap at 45 °C, approximately 20 psi.
12. Samples were reconstituted with 2.5 mL L-Cysteine HCl in MeOH and vortexed for 20 seconds.
13. Samples were transferred to auto-sample vials for LC/MS/MS analysis.

3.3.3 Modifications

Other than instrument parameter optimizations, the following modification was made to the analytical procedure.

1. After fortification, recovery samples were centrifuged at the speed of 4,000 rpm instead of 12,500 g as stated in the method.

3.3.4 Suggestions

1. Due to criticality of the evaporation step in the procedure, one must consider stopping the evaporation in nitrogen evaporator a little earlier and use the technique of rolling the concentration tube and allowing the final volume of solvent to evaporate on its own.
2. In section 4.1 of Bayer Method TD-002-W13-01, specify the time for which the primary stock solutions of thiodicarb and methomyl are stable.
3. In section 4.3 of Bayer Method TD-002-W13-01, clarification is required that two untreated water samples will be fortified with each analyte separately.

3.4 Instrument Operating Parameters

HPLC Conditions			
HPLC Instrument:	Agilent 1200 SL		
HPLC Column:	Ascentis® Express C18, 2.1 mm × 100 mm, 2.7 μm		
Column Temp:	45 °C		
Injection Volume:	5 μL		
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		
Gradient:	Time (min)	Flow (mL/min)	B (%)
	0	0.5	5
	0.5	0.5	5
	0.51	0.5	60
	1.8	0.5	100
	2.5	0.5	100
	2.51	0.5	5
	5	0.5	5

MS/MS Conditions				
Mass Spectrometer	Agilent 6490 Series QQQ			
Ion Source	Electro Spray Ionization (ESI)			
Polarity	Positive			
Scan Type	MRM			
Gas Flow	14 L/min			
Temperature (TEM)	150 °C			
Nebulizer	45 psi			
Sheath Gas Heater	300 °C			
Sheath Gas Flow	12 L/min			
Capillary (V)	3000			
V Charging	1500			
MRM Conditions	Thiodicarb (Quantitation)	Thiodicarb (Confirmatory)	Methomyl (Quantitation)	Methomyl (Confirmatory)
Q1 m/z	355.06	355.06	163.06	163.06
Q3 m/z	88	108	88	105.9
Dwell Time (msec)	100	100	500	300
Frag (V)	380	380	380	380
Collision Energy (CE)	6	10	2	6

3.5 Data Acquisition

Peak integration and peak area count quantitation were performed by MassHunter Quantitative Analysis (version B.04.01) data handling software. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated within LIMS and/or Microsoft® Office Excel spread sheets. Example calculations are presented in Appendix 4.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Prior to conducting the ILV, method control was established. Instrument parameters for the mass spectrometer detector were optimized by infusing standard solutions of the target analytes. The optimized instrument parameters, analyte retention times, instrument detection limits, and linearity were established by injecting a series of calibration standards

4.5 Protocol Changes

Protocol change number 1 was issued to update the experimental design section to instruct the fortification of control water samples should be performed separately in two validation sets due to the rapid degradation of thiodicarb to methomyl.

5.0 CONCLUSIONS

In summary, ADPEN Laboratories successfully and independently validated Bayer Analytical Method TD-002-W13-01 on the second trial.

The method was demonstrated to be suitable for the determination of thiodicarb and methomyl in water at an LOQ of 0.1 ppb and at 10× LOQ. The method is well-written and contains sufficient information to guide the analyst through the procedure for the first time.

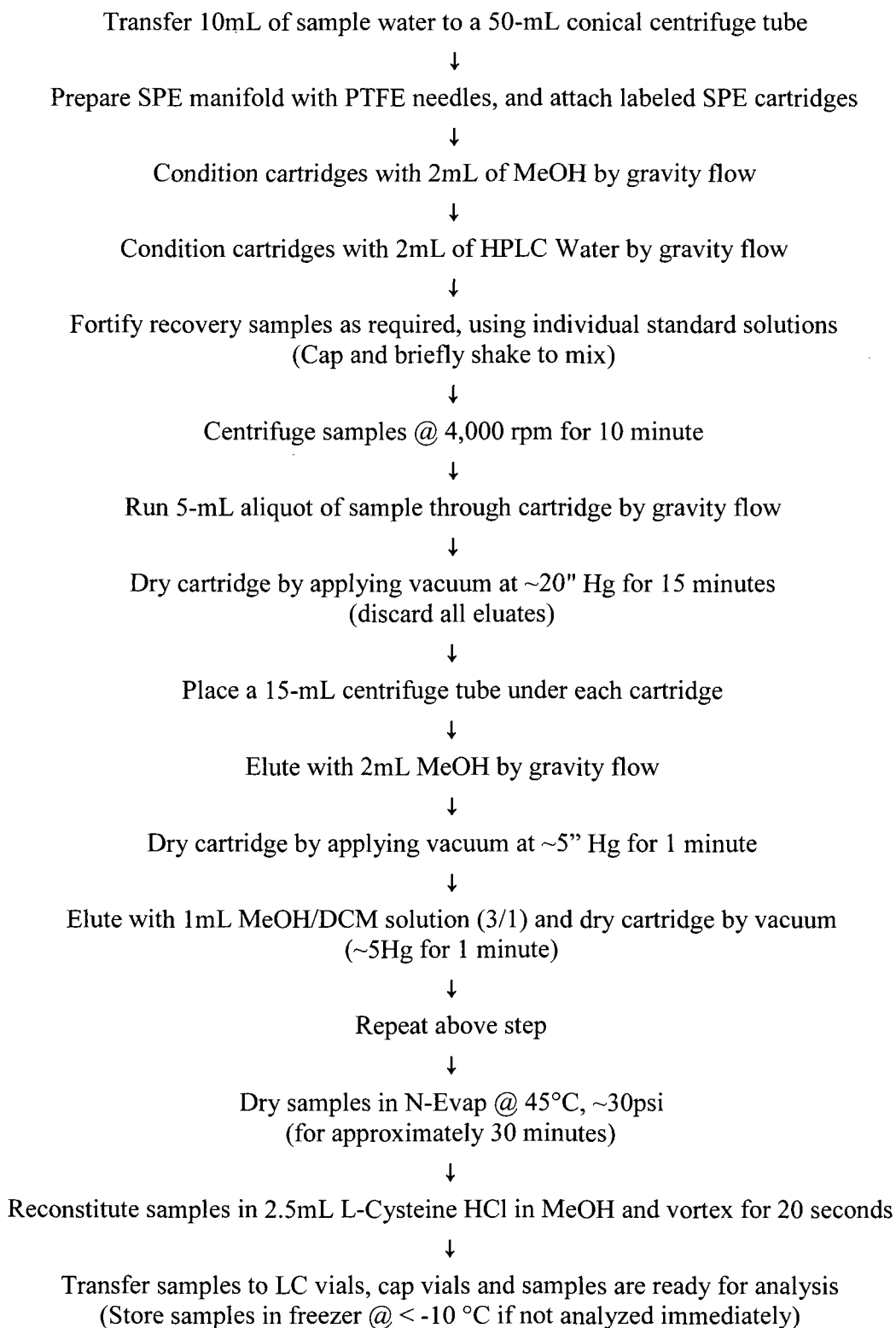
TABLE 1. Flow Diagram of the Analytical Procedure

TABLE 2. Water Fortification Levels

Lab Code	Fortification Level (ppb)	Number of Samples
Analytical set name: WO-13052401 (thiodicarb)		
13052401-MB-1	Method Blank	1
130513004-001C, 130513004-001D	Control	2
13052401-Recovery1-1, 13052401-Recovery1-2, 13052401-Recovery1-3, 13052401-Recovery1-4, 13052401-Recovery1-5	0.1	5
13052401-Recovery2-1, 13052401-Recovery2-2, 13052401-Recovery2-3, 13052401-Recovery2-4, 13052401-Recovery2-5	1.0	5
Analytical set name: WO-13052404 (methomyl)		
13052404-MB-1	Method Blank	1
130513004-001E, 130513004-001F	Control	2
13052404-Recovery1-1, 13052404-Recovery1-2, 13052404-Recovery1-3, 13052404-Recovery1-4, 13052404-Recovery1-5	0.1	5
13052404-Recovery2-1, 13052404-Recovery2-2, 13052404-Recovery2-3, 13052404-Recovery2-4, 13052404-Recovery2-5	1.0	5