Bayer Method DL-005-F10-01
An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS

1.0 SUMMARY

An analytical method was developed to determine the residues of trichlorfon (Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate) and its metabolite DDVP (2,2-dichloroethenyl dimethyl phosphate) in water. The method uses LC/MS/MS with external standards.

The water sample is diluted with acidified acetonitrile and trichlorfon and DDVP analyzed by direct injection by LC/MS-MS. The LOQ of this method is 5.0ng/mL for each analyte.

2.0 BACKGROUND

Trichlorfon is fast-acting insecticide registered by Bayer Environmental Science for control of surface feeding and soil insects including white grubs, mole crickets, sod webworms and cutworms. The analytical method presented in this report is designed to measure residues of trichorfon and its metabolite DDVP using external standards and LC/MS/MS detection.

A summary of the method validation results are presented in Appendix 1 of this report.

3.0 APPARATUS (functional equivalents may be substituted)

- Assorted laboratory ware (including, but not limited to): 100-mL and 2000-mL glass volumetric cylinders
- MicroMan pipettors and tips (Rainen, M50, M250, and M1000) and capillary/ piston sets (Cat. No. CP-50, CP-250, and CP-1000).
- Gastight syringe (Hamilton No. 81265)
- Repeater Plus pipettor and tips (Eppendorf)
- Imtakt Unison UK-C18 3um 75 mm x 3.0 mm column (part no. UK033).
- Shaker table (Eberbach Corp.)
- Finnigan TSQ Quantum Ultra liquid chromatograph/mass spectrometer (LC/MS/MS) equipped with an electrospray interface, Surveyor HPLC pumps, HTS PAL autosampler, and LCquan 2.5 data collection software (Thermo Electron Corporation) **or** an API 4000 chromatograph/mass spectrometer (LC-MS/MS) equipped with an electrospray ionization (ESI) interface, Shimadzu HPLC pumps and a CTC PAL, and Analyst 1.4.1 data collection software (Applied Biosystems)
- Eppendorf CH-30 Column Heater and Eppendorf TC-50 Controller

4.0 REAGENTS AND CONSUMABLES (functional equivalents may be substituted)

- Methanol (MeOH; HPLC Grade; Fisher No. A454-4)
- Water (HPLC Grade; Fisher No. W7-4)
- Formic Acid (Reagent Grade)
- Aqueous 0.1% formic acid solution prepared by adding 1 mL of formic acid to 1000 mL of HPLC grade water
- Glass vials, 60-mL (I-Chem S236-0060)
- Glass jars, graduated, 125-mL (Fisher No. 02-911-455)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)
- Disposable glass Pasteur pipettes, 5 3/4" with bulbs (Fisher No. 13-678-6A)
- Glass jars, 1 gallon (Fisher No. 02-911-918)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)

5.0 PREPARATION OF STANDARD SOLUTIONS

To prepare the standard solutions, reference standards of trichlorfon and DDVP are necessary. These reference standards may be obtained from Bayer CropScience, 17745 South Metcalf, Stilwell, KS 66085. Additional details about these chemicals are given in Appendix 2.

Each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level. All standards should be weighed on a balance accurate to ± 0.01 mg.

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 stock standard solutions should be stored frozen and all secondary standard solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use. Corrections for standard purities should be applied when expressing standard concentrations.

5.1 Primary Stock Standard Solution

Prepare individual 100µg/mL stock solutions of trichlorfon and its metabolite DDVP by transferring about 0.0500 grams of analyte in a 50mL volumetric flask. Dilute to volume with acetonitrile. Store at <-10°C when not in use.

5.2 Fortification Standard Solutions

Prepare individual 1.0µg/mL fortification standards solution of each analyte by taking a 1.0mL aliquot of each of the 100µg/mL stock solution and diluting to 100mL with acetonitrile/0.1% acetic acid aqueous (50/50).

5.3 Calibration Standard Solutions

Prepare a 10µg/mL intermediate mix standard solution of trichlorfon and DDVP by pipetting 10mL each of 100µg/mL stock solutions of trichlorfon and DDVP to a 100mL volumetric flask. Bring the flask to volume with acetonitrile/0.1% acetic acid aqueous (50/50, v/v).

Prepare a 0.1µg/mL intermediate mix standard solution of trichlorfon and DDVP by pipetting 1.0mL of the 10µg/mL mixed stock solution of trichlorfon and DDVP into a 100mL volumetric flask. Bring the flask to volume with acetonitrile/0.1% acetic acid aqueous (50/50, v/v).

Prepare a trichlorfon and DDVP calibration curve standard solutions in acetonitrile / 0.1% acetic acid aqueous (20/80, v/v) as shown in the table below:

Standard Solution Concentration (µg/mL)	Vol of Std Used (mL)	Final Volume (mL)	Spiking Solution Concentration (ng/mL)
10.0	0.20	10.0	200
10.0	0.10	10.0	100
10.0	0.05	10.0	50
0.10	1.00	10.0	10
0.10	0.50	10.0	5.0
0.10	0.10	10.0	1.0
0.10	0.05	10.0	0.5

Further calibration solutions may be prepared as needed.

6.0 PROCEDURE

Appendix 3 shows the analytical scheme for the extraction of trichlorfon and DDVP in water.

Step 1. Measure a 40mL sample into a suitably sized plastic bottle.

Step 2. Fortify the recovery samples as required, fortify one sample with trichlorfon and a second sample with DDVP. (For a fortification at the method LOQ add by pipet 0.04mL of the 1ug/mL fortification solution). As trichlorfon readily degrades to DDVP it is recommended that mixed fortification solutions are not used.

Step 3. Add 10.0mL of 0.25% acetic acid in acetonitrile. Mix the sample well

Step 4. Transfer an aliquot of the extract to a HPLC vial. The sample is ready for LC/MS/MS analysis.

NOTE: If the peak response for any of the analytical targets is outside the range of the calibration curve any further dilution should be performed using an aliquot of the

solution from Step 3, with all dilutions performed acetonitrile / 0.1% acetic acid aqueous (20/80, v/v) and then proceed to step 4.

7.0 ANALYSIS BY LC/MS/MS

7.1 Analytical Procedure

Step 1. Using the recommended procedures listed below, analyze 10µL aliquots of the 0.5, 1.00, 5.00, 10.0, 50.0, 100.0 and 200.0ng/mL standard solutions (these are calibration solution analyses).

Step 2. Analyze 10 µL aliquots of the analytical samples.
Note: Up to 20 sample analyses can be made after the analysis of the standard solutions.

Step 3. Again analyze 10 µL aliquots of the 0.5, 1.00, 5.00, 10.0, 50.0, 100.0 and 200.0ng/mL calibration standard solutions.

Step 4. When necessary, analyze additional samples and standard solutions. Always finish the procedure with the analysis of a set of standard solutions.

7.2 HPLC Conditions

Note: The analyst should optimize chromatographic conditions to obtain satisfactory chromatography. The following recommended conditions were used on both a Thermo Finnigan Ultra and an Applied Biosystems API 4000 instrument:

Solvents: Aqueous 0.1% formic acid solution and 0.1% formic acid in methanol

HPLC column: Imtakt Unison UK-C18 3um 75 mm x 3.0 mm column
Injection volume: 50 µL (Adjust for LC/MS/MS system being used)
Oven Temperature: 40°C

HPLC program:

Time (min)	0.1% formic acid in methanol	Flow rate uL/min
0	60	240
6.0	60	240

Retention Times

Analyte	Approximate Retention Time (minutes)
Trichlorfon	2.8
DDVP	4.2

7.3 Mass Spectrometer Conditions

Note: The analyst should optimize the mass spectrometer conditions to obtain satisfactory system response. The following recommended conditions were used on a Thermo Electron Finnigan TSQ Quantum Ultra and an API 4000 instrument

Instrument ID: Thermo Electron Finnigan TSQ Quantum Ultra		
Component:	Trichlorfon	DDVP
Parent Ion:	257	221
Product Ion:	109	109
Ionization Mode:	ESI	ESI
Polarity:	Positive	Positive
Spray Voltage(V):	4000	4000
Sheath Gas Pressure(psi):	60	60
Aux Gas Pressure(psi):	25	25
Ion Sweep Gas Pressure (psi):	1.0	1.0
API Temp(C):	270	270
Resolution for Q1MS(v):	0.7	0.7
Skimmer offset(v):	18	18
Collision Energy (v):	25	21
Q2 Collision Gas Pressure(mT):	1.3	1.3
Resolution for Q3MS(v):	0.7	0.7

Instrument ID: Applied Biosystems API4000		
Component:	Trichlorfon	DDVP
Parent Ion:	257	221
Product Ion:	109	109
Ionization Mode:	ESI	ESI
Polarity:	Positive	Positive
Declustering Potential (DP):	65	65
Entrance Potential (EP):	10	10
Collision Cell Exit Potential (CXP):	10	10
Collision energy (CE)	27	27
Curtain Gas (CUR)	15	15
Ion Source Gas 1 (GS1)	50	50
Ion Source Gas 1 (GS2)	50	50
Source Temp (TEM)	150	150
Interface Heater (IHE)	On	On
Ion Transfer Voltage (IS)	3000	3000

7.4 Mass Spectrometer Data Collection

Note: The analyst should optimize the mass spectrometer data collection to obtain satisfactory system response. As the HPLC column ages, the retention times of the analytes will change. A standard solution should be analyzed before each set of samples to confirm the data collection parameters.

8.0 CALCULATION OF RESULTS

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Inject samples using the procedure outlined in Section 7.

Trichlorfon and DDVP residues were 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 areas versus the standard concentrations in ng/mL using either Applied Biosystems Analyst Software (Version 1.4.1) or Finnigan LCquan (Version 2.5), computer-programmed data capturing systems. The software uses the MS/MS standard responses to calculate the regression coefficients for slope, M, and intercept, B, for each analytical set with 1/x weighting.

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

After regression coefficients were calculated, the residue in parts per billion was determined. The ng/mL of trichlorfon in the sample was calculated using the following equation,

$$\text{trichlorfon found (ng/mL)} = \frac{(Y-B) \times D}{M}$$

Where Dilution Factor (D) = $\frac{50(V_2)}{40(V_1)}$

Where: V_1 =40mL (Section 6, Step 1)
V_2 =50mL (Section 6, Step 3)

Analyst or LCquan software was used to calculate the amount of trichlorfon in ng/mL in water and the percent recovery for the spiked samples. DDVP residues are determined in a similar fashion.

8.1 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:

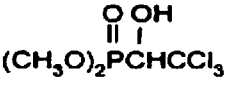
$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where:
- R = ng/mL of target analyte found in fortified sample
- S = ng/mL of target analyte found in control sample, real or apparent
- T = theoretical ng/mL in fortified sample

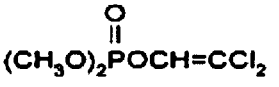
Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at an appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

Appendix 2 Test and Reference Substances

Code Name: Trichlorfon (2,2,2-trichloro-1-hydroxyethyl)phosphonate)
Molecular Formula: $C_4H_8Cl_3O_4P$
Molecular Weight: 257 g/mol



Code Name: DDVP (2,2-dichloroethenyl dimethyl phosphate)Molecular Formula: $C_4H_7Cl_2O_4P$
Molecular Weight: 221 g/mol



The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

Appendix 3 Analytical Scheme for the Analysis of Trichlorfon and DDVP in Water

Measure 40mL of water
↓
Add 10mL of acetonitrile / 0.1% acetic acid
aqueous (20/80, v/v) and analyze by LC/MS/MS