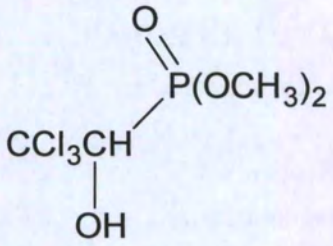
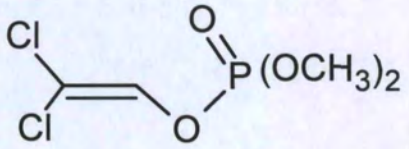
Analytical Reference Standards

Standard Name:	Trichlorfon
Standard Number:	AS029
IUPAC Name:	dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate
CAS Number:	52-68-6
Reference Substance Lot:	0707200401
GLP Purity:	98.9%
Molecular Formula:	$C_4H_8Cl_3O_4P$
Average Mass:	257.44
Molecular Structure:	



Standard Name:	Dichlorvos (DDVP)
Standard Number:	09822
IUPAC Name:	2,2-dichlorovinyl dimethyl phosphate
CAS Number:	62-73-7
Reference Substance Lot:	0310200604
GLP Purity:	99.5%
Molecular Formula:	$C_4H_7Cl_2O_4P$
Average Mass:	220.98
Molecular Structure:	



Other

Upon completion of the study, a copy of the protocol and the final report will be archived at CPS. The original protocol, final report, raw data, correspondence, and other documentation will be transferred to the Bayer CropScience Archives, Bayer CropScience, 2 T.W. Alexander Drive, RTP, North Carolina, 27709.

1.0 EXECUTIVE SUMMARY

Bayer Method DL-004-W10-01, entitled "An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS" [1], was validated successfully in Trial 1. This study was designed to fulfill the requirements of the US EPA Test Guidelines OPPTS 850.7100 [2] and OPPTS 860.1340 [3]. In addition, this study was conducted in compliance with US EPA FIFRA (40 CFR Part 160) GLP standards [4].

The method was successfully validated on the first attempt for trichlorfon and its metabolite dichlorvos (DDVP) in water samples at the limit of quantitation (LOQ) and 10×LOQ concentration levels (0.005 ppm and 0.050 ppm, respectively). Mixed fortification solutions were not used as trichlorfon readily degrades to DDVP.

The relative standard deviations (RSDs) of replicate measurements were less than 20% for each analyte at each fortification level.

The method was performed as written with no major modifications. It took one person approximately 3.0 hours to complete the extraction of one set of 23 samples (one reagent blank, two unfortified matrix control samples, and 20 fortified samples). Time of analysis was approximately 6 hours. To complete one set, including extraction and analysis, took approximately 1.1 days.

This method passed the independent laboratory validation (ILV) on the first attempt with no major modifications.

2.0 INTRODUCTION

The objective of this study was to validate Bayer Method DL-004-W10-01, entitled "An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS" [1]. This method was successful.

This study was designed to fulfill the requirements of the US EPA Test Guidelines OPPTS 850.7100 [2] and OPPTS 860.1340 [3]. In addition, this study was conducted in compliance with US EPA FIFRA (40 CFR Part 160) GLP standards [4].

3.0 MATERIALS AND METHODS

3.1 Test Substance

Standard name:	Trichlorfon
Standard No.:	AS029
IUPAC name:	dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate
CAS number:	52-68-6
Ref. Substance Lot:	0707200401
GLP purity:	98.9%
Expiration date:	18 December 2019
Storage conditions:	Frozen

Standard name:	Dichlorvos (DDVP)
Standard No.:	09822
IUPAC name:	2,2-dichlorovinyl dimethyl phosphate
CAS number:	62-73-7
Ref. Substance Lot:	0310200604
GLP purity:	99.5%
Expiration date:	20 August 2022
Storage conditions:	Frozen

3.2 Test System

The test system used for the validation was a surface/ground water sample obtained from Brandywine Creek near West Chester, Pennsylvania by CPS. The sample was refrigerated (3 ± 3°C) until needed for analysis.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Bayer Method DL-004-W10-01 for Trial 1 (Appendix 5, Section 3: Apparatus and Section 4: Reagents and Consumables). Identical or equivalent apparatus and materials were used.

3.3.1 Equipment and Apparatus

Agilent 1200® HPLC System (Agilent Technologies)
Analytical Balance (Mettler Toledo)
API 4000™ Tandem Mass Spectrometer, MS/MS (Applied Biosystems™)
Manual Micro Pipettor 200 µL (VWR International)
Manual Micro Pipettor 1000 µL (VWR International)
Manual Micro Pipettor 5000 µL (VWR International)
Refrigerator/Freezer (Nor-lake® Scientific)
Ultrasonic Cleaner 5510 (Branson)
Unison UK-C_{18} HPLC Column 75 × 3.0 mm, 3 µm (Imtakt)

3.3.2 Reagents

Acetonitrile (EMD)
Formic Acid (Sigma-Aldrich®)
Glacial Acetic Acid (EMD)
HPLC-grade Water (EMD)
Methanol (EMD)

3.4 Experimental Design

3.4.1 Establishment of the Method

Prior to performing the ILV, the analyte retention times, instrument detection limits, and linearity of instrument responses to a range of analyte concentrations were determined, and the test system was verified as free of interferences at appropriate retention times.

3.4.2 Sample Validation Sets, Fortification, and Extraction Procedure

Sample Validation Sets
Each analytical set consisted of 23 samples: one reagent blank, two untreated controls, five untreated controls fortified with trichlorfon at the Limit of Quantitation (LOQ; 0.005 ppm), five untreated controls fortified with trichlorfon at 10×LOQ (0.050 ppm), five untreated controls fortified with DDVP at the LOQ (0.005 ppm), and five untreated controls fortified with DDVP at 10×LOQ (0.050 ppm).

Data are summarized in Table 1 for Trial 1. Residue data sheets are included in Appendix 1.

Calibration standard solutions (0.001 to 0.100 µg/mL) and blanks were also included in each sample set.

Fortification
The control LOQ and 10×LOQ samples were fortified with 0.040 mL of the appropriate fortification standard solutions of either trichlorfon or DDVP. The fortification standard solutions had a concentration of 5.00 µg trichlorfon or DDVP/mL for the LOQ and a concentration of 50.0 µg trichlorfon or DDVP/mL for the 10×LOQ.

Extraction and Workup
The following extraction steps were followed for each sample.

1. Using a graduated cylinder, added 40 mL of control sample into 50 mL polypropylene tubes. For the reagent blank, HPLC-grade water was used.
2. Added the appropriate amount of fortification solution to the sample.
    a. For the reagent blank and the untreated controls, added 0 mL.
    b. For trichlorfon, added 0.040 mL of 5.00 µg/mL trichlorfon fortification solution for the LOQ samples or 0.040 mL of 50.0 µg/mL trichlorfon fortification solution for 10×LOQ samples.

c. For DDVP, added 0.040 mL of 5.00 µg/mL DDVP fortification solution for the LOQ samples or 0.040 mL of 50.0 µg/mL DDVP fortification solution for 10×LOQ samples.

3. Added 10 mL of 0.25% acetic acid in acetonitrile solution to each sample. The samples were then mixed well.
4. Transferred a 1 mL aliquot of each extract to HPLC vials

3.4.3 Sample Processing and Analysis

The samples were processed and analyzed as described by Bayer Method DL-004-W10-01 [1] for Trial 1.

3.4.4 Fortification and Calibration Standard Solutions Preparation

Trial 1
Primary stock solutions for the two reference standards were prepared by weighing approximately 10.0 mg of each analytical standard into separate tared 20 mL glass scintillation vials and diluting each to volume with acetonitrile using Class A glass 10 mL pipettes. After addition of acetonitrile, sonication was performed on the primary stocks for 5 minutes. For each analyte, one fortification solution was prepared at a concentration of 50.0 µg/mL by adding an appropriate amount of the respective primary stock solution to a 10 mL volumetric flask and diluting to volume with (50:50) 0.1% acetic acid in HPLC-grade water/acetonitrile solution. For each analyte, a second fortification solution was prepared at a concentration of 5.00 µg/mL by measuring 1 mL of the initial respective fortification solution into a 10 mL volumetric flask and diluting to volume with (50:50) 0.1% acetic acid in HPLC-grade water/acetonitrile solution.

A secondary stock solution containing both analytes was prepared at a concentration of 10 µg/mL for each analyte by adding an appropriate amount of each primary stock solution to a 100 mL volumetric flask and diluting to volume with (50:50) 0.1% acetic acid in HPLC-grade water/acetonitrile solution. A second secondary stock solution containing both analytes was prepared at a concentration of 0.100 µg/mL for each analyte by measuring 1 mL of the initial secondary stock solution into a 100 mL volumetric flask and diluting to volume with (50:50) 0.1% acetic acid in HPLC-grade water/acetonitrile solution.

The calibration standard solutions containing both analytes were prepared at concentrations ranging from 0.001 to 0.100 µg/mL for each analyte by adding an appropriate amount of secondary stock solution to a 10 mL volumetric flask and diluting to volume with (80:20) 0.1% acetic acid in HPLC-grade water/acetonitrile solution.

All solutions were stored in a freezer (-15 ± 10°C) when not in use.

3.5 LC-MS/MS Instrumentation

Instrumentation
HPLC System (Agilent 1200®)

Tandem Mass Spectrometry, MS/MS (Applied Biosystems API 4000™)
Software: Applied Biosystems, Analyst® 1.5.1
Unison UK-C_{18} HPLC Column 75 × 3.0 mm, 3 µm (Imtakt)

3.6 Data Acquisition and Reporting

Peak integration was performed by Analyst® software version 1.5.1. The MS detector responses (peak area) for various injected standard concentrations were used to generate an external calibration curve for the analytes of interest. The overall purpose for the external calibration curve was to display acceptable linearity ($r^2 \geq 0.9801$) of the assigned calibration range. The recoveries of the analyte from the fortified samples were calculated by multi-point calibration.

Recovery results were computed for each sample. The equation used for quantification is presented in Appendix 2. A statistical treatment of the data includes the calculation of means, standard deviations (SD), RSDs as percentages (%), and the 95% confidence intervals. All statistics were calculated using Microsoft® Office Excel 2003.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment

The trichlorfon and DDVP transitions from m/z 257.00 to 109.00 and from m/z 221.00 to 109.00, respectively, were used to quantitate the analytes.

Prior to performing the ILV, the analyte retention times, instrument detection limits, and linearity of instrument responses to a range of analyte concentrations were determined, and the test system was verified as free of interferences at appropriate retention times.

4.2 Independent Laboratory Validation Trial Results

Trichlorfon and DDVP eluted as well-resolved chromatographic peaks in (80:20) 0.1% acetic acid in HPLC-grade water/acetonitrile solution with retention times of approximately 2.66 and 4.16 minutes, respectively.

The seven-point calibration curves for trichlorfon and DDVP in (80:20) 0.1% acetic acid in HPLC-grade water/acetonitrile solution were linear over the range of 1.00 to 100 ng/mL and yielded correlation of determinations of $r^2 = 0.9944$ and $r^2 = 0.9948$, respectively.

Representative calibration standard curves for trichlorfon and DDVP in (80:20) 0.1% acetic acid in HPLC-grade water/acetonitrile solution are presented in Figure 1 and Figure 9, respectively. Representative chromatograms of (80:20) 0.1% acetic acid in HPLC-grade water/acetonitrile blank solutions, calibration standard solutions (1.00 and 100 ng/mL), reagent blank samples, untreated control samples, and untreated control samples fortified at LOQ and 10×LOQ for trichlorfon and for DDVP in water are presented in Figure 2 through Figure 8 and Figure 10 through Figure 16, respectively.

4.3 Potential Interferences

No significant interference from the test system was observed.

4.4 Time Required for Analysis

It took one person approximately 3.0 hours to complete the extraction of one set of 23 samples (one reagent blank, two unfortified matrix control samples, and 20 fortified samples). Time of analysis was approximately 6 hours. To complete one set, including extraction and analysis, took approximately 1.1 days.

4.5 Communication with Study Monitor

The study director recognized that the DDVP reference standard was expired and asked the study monitor for further clarification. The study monitor informed CPS that the DDVP has been recertified but the purity was not yet available. The study monitor said the new COA would be accessible within 1 to 2 weeks. The reference standards would be shipped to CPS prior to receipt of the new COA for DDVP. The study director asked for clarification about the test system used for the validation. The study monitor highlighted that any surface/ground water sample on-site will be suitable, otherwise water control can be sampled from a local water source. Due to the current move, no control samples were available for usage. The study director asked if tap water would be a suitable control. The study monitor stated that tap water is to be used as a last resort, and to try to do ILVs on more difficult matrices. The study monitor asked if there were any streams close by for sampling. The study director replied that the Brandywine stream was local. The study director advised the study monitor that the HPLC column (Imtakt Unison UK-C_{18} 3 µm 75 mm × 3.0 mm, PN: UK033) was on back order for at least two weeks and asked if there was an equivalent

column. The study monitor informed the study director that there were no equivalent columns, and the study monitor would search on-site. The study monitor notified the study director that an unused column in an unopened box was located and would be shipped to CPS. The study director emailed the study monitor confirmation of receipt of the column. The study monitor emailed the study director the new COA for DDVP. The study director emailed the study monitor that the ILV was successful on the first attempt and attached the supporting data for review. The study monitor acknowledged that Trial 1 was successful.

5.0 CONCLUSIONS

CPS successfully completed and independently validated the issued version of Bayer Method DL-004-W10-01, entitled "An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS." Bayer Method DL-004-W10-01 (see Appendix 5) was demonstrated to be suitable for the determination of the targeted analytes in water studied at an LOQ of 0.005 µg/mL (0.005 ppm). The method was performed as written with no major modifications. It took one person approximately 3.0 hours to complete the extraction of one set of 23 samples (one reagent blank, two unfortified matrix control samples, and 20 fortified samples). Time of analysis was approximately 6 hours. To complete one set, including extraction and analysis, took approximately 1.1 days.

TABLE 2 HPLC SYSTEM OPERATING PARAMETERS FOR BAYER METHOD DL-004-W10-01 (TRIAL 1)

HPLC System: Agilent Model 1200®
Software: Applied Biosystems, Analyst® 1.5.1
Analytical Column: Unison UK-C_{18} HPLC Column 75 × 3.0 mm, 3 µm (Imtakt)
Column Temperature: 40°C
Injection Volume: 10.0 µL
Run Time: 6.0 minutes

Mobile Phase: (A—Aqueous): 0.1% formic acid in HPLC-grade water
(B—Organic): 0.1% formic acid in methanol
Needle Wash: Flush Port: 15.0 seconds using methanol (prior to injection)

Gradient:

Time (min)	A (%)	B (%)	Flow (µL/min)
0.00	40.0	60.0	240
6.00	40.0	60.0	240

TABLE 3 MS/MS OPERATING PARAMETERS

Tandem Mass Spectrometry System, Applied Biosystems™, API 4000™
Software: Applied Biosystems™, Analyst® 1.5.1

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ion Source:	TurboSpray
Scan Type:	MRM
Polarity:	Trichlorfon: Positive Dichlorvos (DDVP): Positive
Curtain Gas (CUR):	15.00
Temperature (TEM):	150.00
Ion Spray Voltage (IS):	3000.00
Collision Gas (CAD):	7.00
Ion Source Gas 1 (GS1):	50.00
Ion Source Gas 2 (GS2):	50.00
Interface Heater (ihe):	ON
Entrance Potential (EP):	10.00
Transitions Monitored:	
Trichlorfon:	(Q1) 257.00→(Q3) 109.00 m/z
Dichlorvos (DDVP):	(Q1) 221.00→(Q3) 109.00 m/z
Collision Energy (CE):	27.00
Collision Cell Exit Potential(CXP):	10.00
Declustering Potential (DP):	65.00

APPENDIX 2 CALCULATIONS

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Applied Biosystems™, Analyst® software version 1.5.1 using linear regression. Further calculations were performed using the software Microsoft® Office Excel 2003.

The linear equation is expressed as:

$$y = MX + B$$

where y = Native peak area
M = Calibration line slope
X = Concentration of the reference standard in ng/mL
B = Calibration line intercept

Trial 1

By means of the linear equation, the content of trichlorfon and its metabolite, DDVP, in water or recoveries can be calculated as follows:

$$\text{Residue Found (ng/mL)} = \frac{y - B \times D}{M}$$

The dilution factor was calculated using the following equation:

$$\text{Dilution Factor (D)} = \frac{V_2}{V_1}$$

where V_1 = Sample volume (40 mL)
V_2 = Final volume (50 mL)

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