

**INDEPENDENT LABORATORY VALIDATION –BAYER METHOD DL-002-S10-01
FOR TRICHLORFON AND ITS METABOLITES IN SOIL USING LC/MS/MS**

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1.0 SUMMARY

To satisfy US regulatory ILV requirements, a residue analytical method must be validated at an independent laboratory prior to its submission to the appropriate regulatory authority. The purpose of this study is to conduct an independent laboratory validation of the residue analytical method for trichlorfon and its metabolites (dichlorvos [or DDVP], desmethyl DDVP, and dichloroacetic acid [or DCA]), in soil as described in DL-002-S10-01, as written. This study was designed to fulfill the requirements of the U.S. EPA guidelines found in OPPTS 860.1340(c)(6), PR Notice 96-1.

The study was conducted by ABC Laboratories, Inc. of Columbia, Missouri, according to the protocol for Sponsor Study No. MEDLY007, entitled "Independent Laboratory Validation –Bayer Method DL-002-S10-01 for Trichlorfon and its Metabolites in Soil Using LC/MS/MS," and amendment(s).

The method under evaluation has a stated Limit of Quantitation (LOQ) of 250 ng/g for all four analytes in soil. In this study, the method was validated on a composite sample of soil/thatch at the LOQ and 10 × LOQ.

The first method validation trial conducted on soil/thatch was successful for trichlorfon and one of its metabolites (DDVP).

The first and second method validation trials conducted on soil/thatch for two metabolites (desmethyl DDVP and DCA) were unsuccessful due to poor linearity for both analytes at both fortification levels. The first method validation trial was performed as written in the original method and was reinjected with a longer run time. Trial 1 still showed poor linearity believed to be caused by matrix interference. Following discussions with the Sponsor Representative (Method Developer), the second trial included a higher dilution factor and a change in the order of standards and samples. This did not seem to resolve the issue with the matrix interference and still had poor linearity. Following further discussions with the Sponsor Representative (Method Developer), a third method validation trial was conducted using a revised version of the method, Bayer Method DL-002-S10-02, which included the following **minor modification** to the original method: 1) desmethyl DDVP and DCA modified to include the addition of an isotopic internal standard (d_2 -DCA) to both the sample extract and calibration solutions. The third method validation trial was successful with all recoveries for all analytes within criteria range.

No communication, other than the approval of equivalent apparatus, reagents, and techniques; correction of typographical errors; extraction and chromatography issues; clarification of some technical aspects of the methods; and recovery updates between the Sponsor Representative (Method Developer) and Study Director was required.

Interferences were negligible at <30% of the LOQ for the test matrix and each analyte. All individual percent recoveries were within the U.S. EPA guideline acceptance range of 70-120%. Thus, the residue analytical method as described in Bayer Method DL-002-S10-01 was demonstrated to be applicable for the determination of trichlorfon and DDVP, and the residue analytical method as described in Bayer Method DL-002-S10-02, was demonstrated to be applicable for the determination of desmethyl DDVP, and DCA in soil, representative of the matrix for which the methods were intended. The method for the determination of trichlorfon and DDVP in soil via Bayer Method DL-002-S10-01 is identical to that in Bayer Method DL-002-S10-02, demonstrating that both the method versions are applicable.

A single analyst completed sample sets consisting of 23 samples in the course of a typical workday (8 hours) for one group of compounds. To analyze for all compounds, two extraction sets would be completed in two 8 hour days. HPLC LC-MS/MS analysis would be performed overnight, for each set, for approximately 6.5 hours.

2.0 INTRODUCTION

To satisfy US regulatory ILV requirements, a residue analytical method must be validated at an independent laboratory prior to its submission to the appropriate regulatory authority. This study was conducted to fulfill those requirements.

The residue analytical method described in Bayer Method DL-002-S10-01, entitled " An Analytical Method for the Determination of Residues of Trichlorfon and its metabolites in Soil Using LC/MS/MS " ([Appendix 1](#)), is applicable for the quantitation of trichlorfon and DDVP in a variety of soils, and the residue analytical method described in Bayer Method DL-002-S10-02, entitled " An Analytical Method for the Determination of Residues of Trichlorfon and its metabolites in Soil Using LC/MS/MS " ([Appendix 2](#)) is applicable for the quantitation of desmethyl DDVP, and DCA in a variety of soils. In this study, the analytical methods were validated on a representative matrix for which the methods were designed: a composite sample of soil/thatch.

Trichlorfon and its metabolite residues were extracted from the sample by two separate extractions. In the first extraction, a soil sample is fortified with trichlorfon and DDVP. The samples are extracted with acidified methanol/water and shaken. An aliquot of the sample is diluted in acidified acetonitrile and analyzed by LC/MS/MS for trichlorfon and DDVP. In the second extraction, a soil sample is fortified with desmethyl DDVP and DCA. The samples are extracted with aqueous ammonium bicarbonate and shaken. An aliquot of the sample is taken, to which is added the d₂-DCA internal standard, and diluted with acetonitrile (ACN)/0.1% acetic acid (50:50). The solution is then analyzed by LC/MS/MS for desmethyl DDVP and DCA. The limit of quantitation (LOQ) for all analytes was 250 ng/g (250 ppb). The limit of detection (LOD) was estimated to be 83 ng/g (83 ppb).

Except for the minor modifications discussed in Section 3.6 of this report, these methods were performed as written. No communication, other than the approval of equivalent apparatus, reagents, and techniques; correction of typographical errors; extraction and chromatography issues; clarification of some technical aspects of the method; and recovery updates between the Sponsor Representative (Method Developer) and Study Director was required.

3.0 MATERIALS AND METHODS

3.1 Test Substances

The reference analytical standards (test substances) used for this study were:

Trichlorfon:

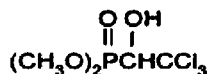
Chemical Name:

IUPAC: dimethyl 2,2,2trichloro-1-hydroxyethylphosphonate

CAS: dimethyl (P)-(2,2,2-trichloro-1-hydroxyethyl)phosphonate

CAS No.: 52-68-6

Chemical Structure:



Molecular Weight: 257.43668

Source: Bayer CropScience

Purity: 98.9%

Lot no.: Not available (Order No.: 9320)
Receipt date: 27 Jan 11
Expiration date: 18 Dec 19
Storage: -20°C

DDVP (Dichlorvos):

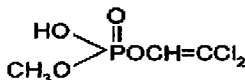
Chemical Name:
CAS: dimethyl O-(2,2-dichlorovinyl) phosphate
CAS No.: 62-73-7
Chemical Structure:



Molecular Weight: 220.97574
Source: Bayer CropScience
Purity: 99.0%
Lot no.: Not available (Order No.: 9321)
Receipt date: 27 Jan 11
Expiration date: 09 Mar 11
Storage: Frozen

Desmethyl DDVP:

Chemical Name:
CAS: methyl O-(2,2-dichlorovinyl) phosphate, 2,2-dichloroethenyl
methyl hydrogen phosphate
CAS No.: Unavailable sodium salt: 13445-62-0
Chemical Structure:



Molecular Weight:
Source: Bayer CropScience
Purity: 99.8%
Lot no.: 0310200603 (24 Jan 11),
Not available (Order No. 9451 (19 Apr 11))
Receipt date: 24 Jan 11 and 19 Apr 11
Expiration date: 09 Mar 21
Storage: Frozen when not in use; ambient before opening

DCA (Dichloroacetic acid):

Chemical Name:

CAS: dichloroacetic acid

CAS No.: 79-43-6

Chemical Structure:



Molecular Weight: 128.94208

Source: Bayer CropScience

Purity: 98.2%

Lot no.: S45929

Receipt date: 27 Jan 11

Expiration date: 15 Sep 12

Storage: Frozen

D₂-DCA or DCA-D₂ (Dichloroacetic acid-d₂) Internal standard:

Chemical Name:

CAS: d₂-dichloroacetic acid

CAS No.: 63403-57-6

Chemical Structure:



Molecular Weight: 130.9544

Source: Bayer CropScience

Purity: NA

Lot no.: Not available (Order No. 9452)

Receipt date: 19 Apr 11

Expiration date: Not provided (Do not use beyond 19 Apr 12)

Storage: Ambient

Trichlorfon, DDVP, desmethyl DDVP, and DCA standards, as well as the D₂-DCA internal standard, were supplied by Bayer CropScience. Information pertaining to the characterization and stability of the test substances is archived by Bayer CropScience. The Certificates of Analysis are included in [Appendix 3](#).

3.2 Test Systems

In this study, the analytical method was validated on the following matrix: a composite sample of soil/thatch. This matrix was chosen as representative of the matrix for which the method was designed – soil.

Control samples of soil/thatch used in the study were provided by the Sponsor Representative via FedEx in ambient temperatures. The soil/thatch control sample for the first trial was not homogenized. All subsequent soil samples were homogenized before being shipped to ABC Laboratories, Inc. by the Sponsor Representative. The success of the original method for trichlorfon and DDVP analytes in un-homogenized soil, demonstrated that soil/thatch homogenization is not considered a factor in the success of

the original method. The revised method used for desmethyl DDVP, and DCA analysis was unsuccessful with both un-homogenized and homogenized soil/thatch, and required an internal standard for successful results, thus demonstrating that soil homogenization is not considered a factor in the success of the revised method. The first soil/thatch samples were immediately placed into limited-access frozen storage (F6-E-W) at a temperature range of $-20 \pm 5^{\circ}\text{C}$ after being received at ABC Laboratories, Inc. The first samples remained in frozen storage until removed for subsampling and analysis. Subsequent soil/thatch samples were placed into a walk-in refrigerator (GXP-435) at a temperature range of 2 to 8°C . The subsequent samples remained in refrigerated storage until removed for subsampling and analysis. The soil/thatch was processed following ABC SOPs.

The samples were assigned unique identification by the laboratory. Additional designations such as "control" and "fortified control," as appropriate, were also assigned by the laboratory.

3.3 Equipment

Equipment used is the same as that specified in the analytical method, except as follows:

Balances:	Mettler Toledo, Model XP205DR, for weighing solid standards (Mettler Instrument Corp., Hightstown, NJ)
	Mettler Toledo, Model BB2440, for weighing soil samples (Mettler Instrument Corp., Hightstown, NJ)
Beakers:	glass, various sizes
Graduated cylinders:	glass, various sizes
HPLC/MS System:	Applied BioSystems/MDS Sciex API 5000 LC/MS/MS system with a Waters Acquity Column Manager UPM, a Waters Acquity Sample Manager UPA, a Waters Acquity Binary Solvent Manager UPB, and a Waters Acquity Sample Organizer. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
	HPLC column: For Trichlorfon and DDVP: Imtakt Unison UK-C18 3 μm , 75 mm x 3.0 mm (part #0JD27A3U). For Desmethyl DDVP and DCA: Sequant Zic Hilic 5 μm , 150 mm x 4.6 mm (part #HX021778)
	HPLC vials: Target Snap Cap Wide opening, clear glass, 2 mL (National Scientific)
	HPLC vial caps: Target National Scientific Kim-Snap closure caps with PTFE/Silicone septa (National Scientific)
Shaker	Eberbach (platform shaker, four different shakers used, all of the same model)

Pipets:	Glass, graduated, serological; various sizes
Pipets, adjustable:	Gilson pipets: 10-100 μL , 1000 μL
Volumetric flasks:	Glass, various sizes

3.4 Reagents and Standards

Reagents and standards used were of equivalent grade as that specified in the analytical method.

3.5 Principles of the Analytical Method

The residue analytical method described in Bayer Method DL-002-S10-01, entitled " An Analytical Method for the Determination of Residues of Trichlorfon and its metabolites in Soil Using LC/MS/MS " ([Appendix 1](#)), is applicable for the quantitation of trichlorfon and DDVP in a variety of soils, and the residue analytical method described in Bayer Method DL-002-S10-02, entitled " An Analytical Method for the Determination of Residues of Trichlorfon and its metabolites in Soil Using LC/MS/MS " ([Appendix 2](#)) is applicable for the quantitation of desmethyl DDVP, and DCA in a variety of soils. The following is a summary of that method:

Trichlorfon and its metabolite residues were extracted from the sample by two separate extractions. In the first extraction, a soil sample is fortified with trichlorfon and DDVP. The samples are extracted with acidified methanol/water and shaken. An aliquot of the sample is diluted in acidified acetonitrile and analyzed by LC/MS/MS for trichlorfon and DDVP. In the second extraction, a soil sample is fortified with desmethyl DDVP and DCA. The samples are extracted with ammonium bicarbonate and shaken. An aliquot of the sample is taken, to which is added the d_2 -DCA internal standard, and diluted with acetonitrile (ACN)/0.1% acetic acid (50:50). The solution is then analyzed by LC/MS/MS for desmethyl DDVP and DCA.

3.6 Modifications, Interpretations, and Critical Steps

The analytical method was run exactly as written except as follows:

Section 5.0 Preparation of Standard Solutions. Includes the preparation of the d_2 -DCA internal standard for use in the desmethyl DDVP and DCA analysis.

Section 6.2, Step 5. Modified to add d_2 -DCA internal standard (25 μL of the 50 $\mu\text{g}/\text{mL}$ solution) into the aliquots used for desmethyl DDVP and DCA analysis.

Section 7.2.5 Desmethyl DDVP and DCA Mass Spectrometer Conditions. Adds the conditions for the d_2 -DCA internal standard

Section 8.0 Calculations of Results. Modified to calculate the desmethyl DDVP and DCA residues by internal standard linear regression analysis.

Appendix 1 Test and Reference Substances. Adds the d_2 -DCA internal standard to the list of references and appropriate information.

All of the above listed modifications are stated with specificity in the revised version of the method, Bayer Method DL-002-S10-02 ([Appendix 2](#)).

3.7 Instrumentation

The quantitative analysis of trichlorfon and its metabolites was performed using Waters Acquity HPLC units coupled to an Applied BioSystems/MDS Sciex API 5000 LC/MS/MS system. The system parameters are shown in the tables below. Peak area was used for quantitation.

HPLC Conditions (Trichlorfon and DDVP):

System:	Applied BioSystems/MDS Sciex API 5000 LC/MS/MS system with a Waters Acquity Column Manager UPM, a Waters Acquity Sample Manager UPA, a Waters Acquity Binary Solvent Manager UPB, and a Waters Acquity Sample Organizer. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.			
Column:	Imtakt Unison UK-C18 3 μ m, 75 mm x 3.0 mm			
Column Temperature:	30 °C			
Injection Volume:	1.0 μ L (Trichlorfon and DDVP) 2.0 μ L (desmethyl DDVP)			
Autosampler Temperature:	10 °C			
Flow Rate:	0.3 mL/minute			
Mobile Phase:	A: 0.1% Formic acid in water B: Acetonitrile			
Mobile Phase Conditions:	<u>Time</u>	<u>%A</u>	<u>%B</u>	<u>Flow (mL/min)</u>
	0.00	75.0	25.0	0.3
	2.00	75.0	25.0	0.3
	3.50	60.0	40.0	0.3
	5.50	5.0	95.0	0.3
	7.00	5.0	95.0	0.3
	7.10	75.0	25.0	0.3
	10.00	75.0	25.0	0.3
Retention Times:	Trichlorfon	~3.2 minutes		
	DDVP	~5.6 minutes		
Total Run Time:	~10 minutes			

HPLC Conditions (Desmethyl DDVP, DCA and D₂-DCA Internal Standard):

System: Applied BioSystems/MDS Sciex API 5000 LC/MS/MS system with a Waters Acquity Column Manager UPM, a Waters Acquity Sample Manager UPA, a Waters Acquity Binary Solvent Manager UPB, and a Waters Acquity Sample Organizer. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.

Column: Sequant Zic Hilic 5 µm, 150 mm x 4.6 mm

Column Temperature: Ambient

Injection Volume: 1.0 µL

Autosampler Temperature: 10 °C

Flow Rate: 0.3 mL/minute

Mobile Phase: A: 0.1% Formic acid in water

B: Acetonitrile

	<u>Time</u>	<u>%A</u>	<u>%B</u>	<u>Flow (mL/min)</u>
	0.00	20.0	80.0	0.3
	4.00	35.0	65.0	0.3
	4.10	50.0	50.0	0.3
Mobile Phase Conditions:	5.00	50.0	50.0	0.3
	5.10	50.0	50.0	0.3
	6.00	50.0	50.0	0.3
	6.10	20.0	80.0	0.3
	9.00	20.0	80.0	0.3
	12.00	20.0	80.0	0.3

Desmethyl DDVP ~3.5 minutes

DCA ~3.9 minutes

d₂-DCA ~3.9 minutes

Total Run Time: ~12 minutes

The detection method utilized was LC-MS/MS employing electrospray (TIS) interface in the positive mode on a triple quadrupole instrument. The instrument was tuned by infusing the analytes into a TIS (turbo ion spray) source, then creating a tune file to maximize the response of each analyte using the TIS source. The acquisition method was adjusted to maximize the response of the fragment ions detected. The ion transitions for each analyte are shown in the table below:

MS Conditions:

System: Applied BioSystems/MDS Sciex API 5000 LC/MS/MS system

Analytes Monitored	Ions Monitored (AMU)	Declustering Potential (volts)	Collision Energy (volts)	Dwell Time (milli-seconds)	EP (volts)	CXP (volts)	Acquisition Timing (minutes)
Trichlorfon	257 → 109	65	50	200	10	10	0-10
DDVP	221 → 109	75	50	200	10	10	0-10
Desmethyl DDVP	205 → 110	-50	-36	300	-9	-4	0-12
DCA	127 → 83	-30	-14	300	-10	-7	0-12
d ₂ -DCA	128 → 84	-30	-14	300	-10	-7	0-12

Additional detector settings are shown in the table below:

Trichlorfon and DDVP

<u>Parameter</u>	<u>Setting</u>
Acquisition Mode:	MRM
Ionization Mode:	positive (+)
Source Temp.:	500 °C
Nebulizer (GS1):	60
Auxillary Gas (GS2):	50
Curtain Gas:	6
CAD Gas:	15
Ion Spray Voltage:	5500

Desmethyl DDVP, DCA, and d₂-DCA

<u>Parameter</u>	<u>Setting</u>
Acquisition Mode:	MRM
Ionization Mode:	negative (-)
Source Temp.:	150 °C
Nebulizer (GS1):	40
Auxillary Gas (GS2):	40
Curtain Gas:	40
CAD Gas:	9
Ion Spray Voltage:	-4500

The instrument was operated in the MS/MS (MRM) positive ion mode for quantitative analysis. Single transition chromatograms for each analyte were integrated and the peak areas used for quantitation. Quantitation was performed using a single transition for each analyte.

For each analytical run, a seven-point standard curve was prepared by injecting constant volumes of standard solutions of a mixture of two analytes, trichlorfon and DDVP or desmethyl DDVP and DCA. Desmethyl DDVP and DCA also contained an internal standard, d₂-DCA, and the ratio of the analyte peak to the internal standard peak area was used to determine the residue. Seven curve check standards were typically injected before and after all of the sample injections.

3.8 Calculations

Calculations were performed as directed by the method. A validated software application was used to create a standard curve based on linear regression. Linear regression was monitored to support the response linearity of the mass spectrometer detector. The regression functions were used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak response) to demonstrate that a linear relationship exists between analyte concentration and peak response, and that a response factor approach to calculation was appropriate.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
x	=	ng/mL found for peak of interest
m	=	slope
b	=	y-intercept

Equations for Trichlorfon and DDVP

The calculations for ppb found and percent recovery (for fortified samples) were:

The amount of analyte (in ppb) found in the sample was calculated according to the following equation:

$$\text{ppb Found} = \frac{\text{ng/mL Found} \times \text{Aliquot Factor} \times \text{Final Volume} \times \text{Dil.Fac.}}{\text{Sample weight}}$$

where:

ng/mL Found = $\frac{(\text{peak area} - b)}{m}$
 peak area = peak area response of the analyte in sample extract

Aliquot Factor = $\frac{\text{Extraction Volume (mL)}}{\text{Aliquot Volume (mL)}}$

Extraction Volume = The mL volume of the initial extract (400 mL for soil)

Aliquot Volume = The mL volume aliquot of initial extract processed through the procedure (0.05 mL for soil)

Final Volume = The mL volume of the final sample extract submitted to HPLC (typically 1.0 mL)

Dil. Fac. = The magnitude of dilution required to bracket the response of the sample within the standard curve responses. No dilution = HPLC dilution factor of 1

Sample Weight = grams of sample extracted (200 g for soil)

Percent recovery of fortified samples (procedural fortifications) was determined using the following equation:

$$\% \text{ Recovery} = \frac{\text{ppb Found in Fortified Sample} - \text{ppb Found in Control}}{\text{ppb Added}} \times 100$$

Example Calculations for Trichlorfon and DDVP

Trichlorfon and DDVP were calculated for soil as follows:

66769-003, Trichlorfon, soil, Set #1, Fortified Control @ 250 ppb:

Sample peak

response = 75841

m = 11738.55

b = 887.0353

ng/mL Found = $(75841 - 887.0353) / 11738.55 = 6.38528$ ng/mL

Aliquot Factor = $\frac{400 \text{ mL}}{0.05 \text{ mL}} = 8000$

ppb Found = $\frac{6.38528 \text{ ng/mL} \times 8000 \times 1.0 \text{ mL} \times 1}{200 \text{ g}} = 255.411$ ppb, reported as 255 ppb

% Recovery = $\frac{255.411 \text{ ppb} - 0 \text{ ppb}}{250 \text{ ppb}} \times 100 = 102\%$

Equations for DCA

The calculations for ppb found and percent recovery (for fortified samples) were:

The amount of DCA (in ppb) found in the sample was calculated with an internal standard (d_2 -DCA) according to the following equation:

$$\text{ppb Found} = \frac{\text{ng/mL Found} \times \text{Aliquot Factor} \times \text{Final Volume} \times \text{Dil.Fac.}}{\text{Sample weight}}$$

where:

ng/mL Found = (IS ratio - b)/m

IS ratio = sample peak area/IS peak area

sample peak area = peak area response of the analyte in sample extract; IS peak area = peak area response of the internal standard in sample extract

$$\text{Aliquot Factor} = \frac{\text{Extraction Volume (mL)}}{\text{Aliquot Volume (mL)}}$$

Extraction Volume = The mL volume of the initial extract (400 mL for soil)

Aliquot Volume = The mL volume aliquot of initial extract processed through the procedure (1 mL for soil)

Final Volume = The mL volume of the final sample extract submitted to HPLC (typically 25 mL)

Dil. Fac. = The magnitude of dilution required to bracket the response of the sample within the standard curve responses. No dilution = HPLC dilution factor of 1

Sample Weight = grams of sample extracted (200 g for soil)

Percent recovery of fortified samples (procedural fortifications) was determined using the following equation:

$$\% \text{ Recovery} = \frac{\text{ppb Found in Fortified Sample} - \text{ppb Found in Control}}{\text{ppb Added}} \times 100$$

Example Calculations for DCA

DCA was calculated for soil as follows:

66769-077, DCA, soil, Set #4, **Fortified Control @ 250 ppb**:

Sample peak
response = 665570

IS peak
response = 4467285

IS ratio = $665570/4467285 = 0.14899$

m = 0.0273259

b = 0.008575698

ng/mL Found = $(0.14899 - 0.008575698) / 0.0273259 = 5.13842 \text{ ng/mL}$

Aliquot Factor = $\frac{400 \text{ mL}}{1 \text{ mL}} = 400$

$$\text{ppb Found} = \frac{5.13842 \text{ ng/mL} \times 400 \times 25 \text{ mL} \times 1}{200 \text{ g}} = 256.921 \text{ ppb, reported as 257 ppb}$$

$$\% \text{ Recovery} = \frac{256.921 \text{ ppb} - 0 \text{ ppb}}{250 \text{ ppb}} \times 100 = 103\%$$

Equations for Desmethyl DDVP

The calculations for ppb found and percent recovery (for fortified samples) were:

Desmethyl DDVP rapidly converts to DCA due to the soil matrix effect, and thus the DCA found, expressed as desmethyl DDVP equivalents, must be added to the desmethyl DDVP found to attain proper residue results. The amount of desmethyl DDVP (in ppb) found in the sample was calculated with an internal standard (d_2 -DCA) added to the DCA found in the sample in desmethyl DDVP equivalents (in ppb), according to the following equation:

$$\text{ppb Found} = \frac{\text{ng/mL Found} \times \text{Aliquot Factor} \times \text{Final Volume} \times \text{Dil. Fac.}}{\text{Sample weight}} + \text{DCA eq}$$

where:

$$\text{ng/mL Found} = \frac{(\text{IS ratio} - b)/m}{\text{IS ratio} = \text{sample peak area}/\text{IS peak area}}$$

sample peak area = peak area response of the analyte in sample extract; IS peak area = peak area response of the internal standard in sample extract

$$\text{Aliquot Factor} = \frac{\text{Extraction Volume (mL)}}{\text{Aliquot Volume (mL)}}$$

$$\text{Extraction Volume} = \text{The mL volume of the initial extract (400 mL for soil)}$$

$$\text{Aliquot Volume} = \text{The mL volume aliquot of initial extract processed through the procedure (1 mL for soil)}$$

$$\text{Final Volume} = \text{The mL volume of the final sample extract submitted to HPLC (typically 25 mL)}$$

$$\text{Dil. Fac.} = \text{The magnitude of dilution required to bracket the response of the sample within the standard curve responses. No dilution = HPLC dilution factor of 1}$$

$$\text{Sample Weight} = \text{grams of sample extracted (200 g for soil)}$$

$$\text{DCA eq} = \text{DCA found in the desmethyl DDVP fortified sample as presented in the calculation above, then converted to desmethyl DDVP equivalents by}$$

multiplying the ratio of the molecular weight of desmethyl DDVP to the molecular weight of DCA. The calculation is presented below:

$$\text{DCA ppb found} \times \frac{\text{molecular weight of desmethyl DDVP (206.949 g/mol)}}{\text{molecular weight of DCA (128.94208 g/mol)}}$$

Percent recovery of fortified samples (procedural fortifications) was determined using the following equation:

$$\% \text{ Recovery} = \frac{\text{ppb Found in Fortified Sample} - \text{ppb Found in Control}}{\text{ppb Added}} \times 100$$

Example Calculations for Desmethyl DDVP

Desmethyl DDVP was calculated for soil as follows:

66769-082r, Desmethyl DDVP, soil, Set #4, **Fortified Control @ 2500 ppb:**

$$\begin{array}{l} \text{Sample peak} \\ \text{response} = \end{array} \quad 97488$$

$$\begin{array}{l} \text{IS peak} \\ \text{response} = \end{array} \quad 11092148$$

$$\text{IS ratio} = \quad 97488/11092148 = 0.00879$$

$$m = \quad 0.04158237$$

$$b = \quad 0.009622492$$

$$\text{ng/mL Found} = (0.00879 - 0.009622492) / 0.04158237 = 0 \text{ ng/mL (number found was less than 0, and thus treated as 0)}$$

$$\text{Aliquot Factor} = \frac{400 \text{ mL}}{1 \text{ mL}} = 400$$

$$\text{Desmethyl DDVP ppb Found} = \frac{0 \text{ ng/mL} \times 400 \times 25 \text{ mL} \times 1}{200 \text{ g}} = 0 \text{ ppb}$$

$$\begin{array}{l} \text{DCA ppb} \\ \text{Found} = \end{array} \quad 1451.49418 \text{ ppb (using equation for DCA as presented above)}$$

$$\text{DCA eq} = \quad 1451.49418 \text{ ppb} \times \frac{206.949 \text{ g/mol}}{128.94208 \text{ g/mol}} = 2329.614 \text{ ppb}$$

$$\text{Desmethyl DDVP ppb Found} = 0 \text{ desmethyl DDVP ppb} + 2329.614 \text{ DCA eq ppb, reported as 2330 ppb}$$

$$\% \text{ Recovery} = \frac{2330 \text{ ppb} - 0 \text{ ppb}}{2500 \text{ ppb}} \times 100 = 93\%$$