syngenta

Chlorothalonil

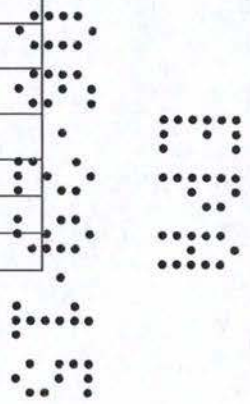
Analytical Method (GRM005.08A) for Residue Determination of Chlorothalonil in Soil by LC-MS/MS

Analytical Method

DATA REQUIREMENT(S): EPA OCSPP 850.6100
EC SANCO/3029/99 rev 4
EC SANCO/825/00 rev 8.1

Abbreviations and Symbols

Abbreviation	Definition
°C	degrees Celsius or Centigrade
amu	atomic mass unit
CAS	Chemical Abstract Services
cm	centimeter
EPA	Environmental Protection Agency (U.S.)
EC	European Commission
EU	European Union
g	gram
GRM	Global Residue Method
HPLC	high performance liquid chromatography
i.d.	internal diameter
IUPAC	International Union of Pure and Applied Chemistry
kg (or Kg)	kilogram
L	liter
LC-MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
m	meter
MeOH	methanol
µg	microgram
µL	microliter
µm	micrometer
mg	milligram
mL	milliliter
mm	millimeter
mmol	millimole
min	minute
mol	mole
ms	millisecond
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
m/z	mass to charge ratio



Abbreviation	Definition
N/A (or n/a)	not applicable
ND (or nd)	not detectable (below limit of detection)
ng	nanogram
No.	number
OES	Occupational Exposure Standards
OECD	Organization for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ppb	parts per billion or micrograms per kilogram or micrograms per liter
ppm	parts per million or milligrams per kilogram or milligrams per liter
pg	picogram
R (or r)	correlation coefficient
R^2 (or r^2)	coefficient of determination or square of correlation coefficient
RSD	relative standard deviation
Rt (or RT)	retention time
s (or sec)	second
SD (or STDEV)	standard deviation
SHC	Syngenta Hazard Category
SPE	Solid Phase Extraction
UPW	ultrapure water
V	volt
Vol (or vol)	volume

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM005.08A is suitable for the determination of chlorothalonil (Figures 1) in soil. The limit of quantification (LOQ) of the method has been established at 5 ppb (5 µg/kg) for analysis of chlorothalonil in soil.

This method satisfies US EPA guidelines EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

1.2 Method Summary

Typically, a sub-sample (20 g) of soil is extracted with 100 mL of acidified acetone by shaking at room temperature for two hours. An aliquot of the soil extract is purified with a Bond Elut C18 solid phase extraction (SPE) cartridge. The SPE final eluent is collected and concentrated by a gentle stream of N_2 to yield the sample final fraction. The final fraction is analyzed for chlorothalonil using LC-MS/MS with negative ESI.

The LOQ of the method has been established at 5 ppb (5 µg/kg) for analysis of chlorothalonil in soil.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare individual 100 µg/mL stock solutions for chlorothalonil by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient individual analytical standards (purity corrected) into an amber "Class A" volumetric flask (100 mL). Dilute to the mark with MeOH to give a 100 µg/mL stock solutions of chlorothalonil.

Alternatively, the appropriate volume of methanol to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P	=	Standard purity in decimal form (P(%)/100)
V	=	Volume of MeOH required
W	=	Weight, in mg, of the solid analytical standard
C	=	Desired concentration of the final solution, (µg/mL)
1000	=	Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions containing chlorothalonil should be prepared by serial dilution in MeOH. It is recommended that the following mixed fortification solutions are prepared for fortification purposes: 1.0 µg/mL and 0.1 µg/mL.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

No significant suppression or enhancement of the instrument responses (at 1.0 ng/mL concentration level) for chlorothalonil has been observed in the soil types tested using the procedures described in Section 3.0 during method development/validation phase using LC-MS/MS technique. Calibration standards should be prepared by diluting the 1.0 µg/mL fortification standard with 20/80 (v/v) MeOH/0.1 % formic acid in ultrapure water. It is recommended that the following calibration standard solutions are prepared: 20, 10, 5, 2, 1, 0.5 and 0.2 ng/mL.

Any matrix effects observed may be compensated for by use of matrix-matched standards at the discretion of the study director, or by dilution of the final sample with 20/80 (v/v) MeOH/0.1% formic acid in ultrapure water should instrument sensitivity permits.

Calibration curves should be generated to quantify residues of chlorothalonil in a sample set using 1/x weighing factor. Standards over an appropriate concentration range should be prepared in a manner similar to description above.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in a refrigerator or freezer when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of three months for chlorothalonil in MeOH is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S. G. Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent Hazards

	Acetonitrile	MeOH	Formic acid	Sulfuric acid	Acetone
Harmful Vapor	✓	✓	✓	✓	✓
Highly Flammable	✓	✓	✗	✗	✓
Harmful by Skin Absorption	✓	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓	✓
Causes severe burns	✗	✗	✓	✓	✗
Syngenta Hazard Category (SHC)	SHC-C, S	SHC-C, S	SHC-C, S	SHC-C, S	SHC-B, S
OES Short Term (mg/m^3)	105	310	N/A	N/A	3,560
OES Long Term (mg/m^3)	70	260	9	1	1,780

N/A not known; * Based on NH_3

At present there are insufficient data available to assign a Syngenta Hazard Classification for chlorothalonil. It should be treated as a category SHC-D compounds until further information indicates otherwise. The Syngenta Hazard Category scale rates highly toxic

chemicals as category SHC-E and non-toxic chemicals as category SHC-A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form in Appendix 4.

3.1 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.2 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included with each sample set. To each pre-weighed control soil sample, add the appropriate amount of standard solution containing chlorothalonil in MeOH. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analyzed with each sample set.

3.3 Extraction

a) Weigh a representative sub-sample of soil (20 g) into a Nalgene plastic bottle (250 mL). Fortify samples with the fortification solution in MeOH as required at this point. At least one untreated control and two control samples fortified with known amounts of analytes of interest should be analyzed with each sample set, using the same procedure, to verify method performance. The volume of the fortification solution added should stay within 0.5 – 1 mL. Allow fortified control samples to equilibrate for at least 5 minutes before proceeding to the extraction.

b) Add 100 mL of freshly prepared extraction solvent, *i.e.* acidified acetone (freshly prepared by mixing 1,540 mL acetone with 60 mL of 50% H_2SO_4 in ultrapure water) into the extraction bottle and cap.

c) Place the bottle (horizontal orientation) on a mechanical shaker and shake at a speed (typically ~ 300 cps) that visibly agitates the samples vigorously for 2 hours at room temperature.

d) Centrifuge samples at approximately 3500 rpm with refrigeration at 10°C (or at a speed that visibly separates the solid sample from the supernatant) for about 10 minutes.

e) Decant the supernatant to a clean brown Nalgene plastic bottles (125 mL);

Note: With some soils, particularly those with high clay contents, the solution may still be visibly cloudy even after centrifugation. This is normal and will not affect results.

f) Mix the final soil extract well prior to solid phase extraction (SPE) procedures. Store the extract in a refrigerator (~4 °) if the next step cannot be conducted immediately.

3.4 Solid Phase Extraction (SPE) Clean-up Procedure

The sample cleanup and concentration are accomplished by the use of Agilent Bond Elut C18 solid phase extraction (500 mg, 6-mL) cartridge. Avoid the cartridges from drying during the entire process unless specified. Allow one solvent to pass through the SPE to approach the bed frit before adding the next solvent. The flow rate should be kept at a rate of less than 20 drops per minute (approximately 1 mL/min). Flow efficiency can be improved by controlled vacuum on the SPE extraction box or controlled positive pressure on the SPE cartridge, however, **gravity flow** is highly recommended for the sample loading and final elution. The SPE procedures are described as below:

a) Precondition a Bond Elut C18 SPE cartridge by passing 3 mL of MeOH through the cartridge under slight vacuum or by gravity and discarding the eluent;

b) Pass 3 mL of ultrapure water under slight vacuum or by gravity and discard the eluent;

c) Transfer an aliquot (2 mL) from the extract(Section 3.3(f)) into a clean disposable polypropylene centrifuge tube (15 mL) and dilute the extract with 10 mL of ultrapure water. Ensure to mix the sample well;

d) Load the diluted sample from Section 3.4(c) onto the preconditioned C18 SPE cartridge; portion-wise and quantitatively. Slight positive pressure or vacuum may be applied if needed; however, the flow rate should be less than 20 drops per minutes (approximately 1 mL/min). Do not allow the SPE cartridge to go dry and discard the eluent;

e) Rinse the used polypropylene centrifuge tube with 3 mL of 50/50 (v/v) MeOH/ultrapure water and pass the rinse through the SPE cartridge. Do not allow the SPE cartridge to go dry and discard the eluent;

f) Pass 4 mL of 50/50 (v/v) MeOH/ultrapure water through the SPE cartridge for cleanup and discard the wash;

g) Repeat Section 3.4 (f) for two more times, i.e. a total of three portions of 50/50 (v/v) MeOH/ultrapure water (4 mL) for cleanup, and discard the eluents;

h) Add 5 mL of acetonitrile to the SPE to elute chlorothalonil residues from the SPE cartridge and collect the eluent into a clean polypropylene centrifuge tube (15 mL). Let the SPE cartridge to go dry this time;

i) Add 1 mL of 0.1% formic acid in ultrapure water to the sample and as a keeper and mix well.

j) Evaporate the resulting solution to a volume just less than 1 mL (but not dryness and avoid extensive drying time) under a gentle stream of N_2 at a bath temperature of approximately 40°C.

k) Re-constitute the sample to 2 mL (adjust to appropriate final volume as needed) with 40/60 (v/v) MeOH/0.1% formic acid in ultrapure water to product the sample final fraction;

l) Sonicate the sample for 5 minutes and transfer~ 1 mL of the sample final fraction into a HPLC injection vial for LC-MS/MS ESI Ion Source analysis. See Section 4.1 – 4.3 for LC-MS/MS ESI Ion Source conditions/parameters for analysis of chlorothalonil and use the calibration standards as described in Section 2.3.3.

3.5 Optional SPE Clean-up Procedure for LC-MS/MS with APPI Ion Source

The sample cleanup and concentration are accomplished by the use of Agilent Bond Elut C18 solid phase extraction (500 mg, 6-mL) cartridge. In general, avoid cartridges from drying during the process unless specified. Allow one solvent to flow through the SPE (no liquid layer on top of bed) before adding the next solvent. The flow rate should be kept at a rate of less than 20 drops per minute (approximately 1 mL/min). Flow efficiency can be improved by controlled vacuum on the SPE extraction box or controlled positive pressure on the SPE cartridge, however, **gravity flow** is highly recommended for the sample loading and final elution. The SPE procedures are described as below:

a) Precondition the SPE cartridges as follows:
   1. 3 mL of MeOH, one time.
   2. 3 mL of ultrapure water, one time.

b) Transfer an aliquot (2 mL) from the extract(Section 3.3(f)) into a clean disposable polypropylene centrifuge tube (15 mL) and dilute the extract with 10 mL of ultrapure water. Ensure to mix the sample well.

c) Load the sample from Section 3.4(b) onto the preconditioned C18 SPE cartridge; portion-wise and quantitatively. Slight positive pressure or vacuum may be applied if needed; however, the flow rate should be less than 20 drops per minutes (approximately 1 mL/min). Do not allow the SPE cartridge to go dry and discard the eluents.

d) Rinse Wash the SPE cartridge as follows and discard the washes.
   1. Rinse the samples tube with 1 mL of 20/80 (v/v) MeOH/ultrapure water and transfer the rinsate to the SPE cartridge;
   2. 2 x 3 mL of 20/80 (v/v) MeOH/ultrapure water;

e) Elute the cartridge with 4 mL of MeOH and collect into a clean receiving tube.

f) Add 1 mL of 0.1% formic acid in ultrapure water as a keeper and mix well.

g) Evaporate the resulting solution to a volume less than 1 mL (but not dryness and avoid extensive drying time) under a gentle stream of N_2 at a bath temperature of approximately 40°C.

h) Re-constitute the sample to 2.0 mL (adjust to appropriate final volume as needed) with 0.1% formic acid in 50/50 (v/v) MeOH/ultrapure water.

i) Vortex and transfer into an HPLC vial for LC-MS/MS APPI Ion Source analysis. See Sections 4.4 – 4.7 for LC-MS/MS APPI Ion Source conditions/parameters for analysis of chlorothalonil and use the calibration standards as described in Section 2.3.3.

3.6 Experimental Precautions

a) The SPE procedures have been developed using cartridges and media material from the stated manufacturer. Similar material or cartridges from other manufacturers may be used. In all cases however, it is strongly recommended that the elution profile of the chosen batch of cartridges is checked prior to commencing analysis to assess any variation in manufacturers' products and between batches.

b) Bottled HPLC grade ultrapure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system.

c) To prevent contamination of instrument and to minimize possible carry-over issues using LC-MS/MS, it strongly recommended to inject 20/80 (v/v) MeOH/0.1% formic acid in ultrapure water between standards or/and samples to clear any observed carry-over. It is also recommended that high level recoveries (> 50 ppb) and samples with expected residues greater than 50 ppb should be diluted so that the final individual analyte concentration does not exceed 20 ng/mL.

3.7 Time Required for Analysis

The methodology is normally performed with a batch of 13 samples. One skilled person can complete the extraction procedures in one day (8 hour working period) for LC-MS/MS analysis. The analytical sequences were normally carried out with overnight arrangement to maximize the lab output.

3.8 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

4.1 LC-MS/MS Instrument Description

Pump	:	Waters Acquity UPLC® system (I Class) with Sample Manager and Column Manager
Detector	:	Applied Biosystems Sciex API 5500 Qtrap mass spectrometer with Analyst TM software version 1.6.2

4.2 LC Chromatographic Conditions for Analysis of Chlorothalonil

Column	:	Zorbax SB-Aq, 4.6 x 50 mm, 1.8 µm
Column Oven Temperature	:	Ambient
Injection volume	:	20 µL
Stop Time	:	14 minutes
Injection protocol	:	Analyze calibration standard after 4 to 5 sample Injections
Sample Tray Temperature	:	10°C
Mobile phase	:	Solvent A: 0.1 mM ammonium acetate in ultrapure water Solvent B: MeOH

Mobile Phase Composition

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0	80	20	0.6
0.5	80	20	0.6
8	5	95	0.6
12	10	95	0.6
12.1	80	20	0.6
15	80	20	0.6

Note: Under these conditions the retention time of Chlorothalonil is ~ 7 minutes.

4.3 Mass Spectrometer Conditions/Parameters

Interface	:	TurboIonSpray
Polarity	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 25 (arbitrary units)
Temperature (TEM)	:	650°C
Ionspray voltage	:	-4200
Collision gas setting (CAD)	:	Medium
Gas 1 (GS1)	:	Air set at 45 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Operating Parameters:

MS/MS Transitions

Analyte	MS/MS Transition*	Dwell (ms)	DP	EP	CE	CXP	RT (min.)
Chlorothalonil	ESI Negative						
Primary	244.8 → 181.9	50	-65	-10	-38	-9	7
Confirmatory	244.8 → 209.9	50	-65	-10	-33	-9	7
Confirmatory (alternative)	244.8→ 174.9	50	-65	-10	-38	-11	7

Typical chromatograms are shown in the Figures Section.

4.4 Optional LC-MS/MS Instrument Description

HPLC System	:	Surveyor Plus LC System – A binary solvent system equipped with MS Pump Plus
Autosampler	:	Surveyor MS Plus
Detector	:	Thermo Electron TSQ Quantum Ultra mass spectrometer with Xcalibur™ Software
Collision Gas	:	Purified Argon in compressed cylinder
Gas Supply	:	House Nitrogen supply

An integrated Thermo Electron TSQ Quantum Ultra mass spectrometer was used to establish and validate the method for chlorothalonil. The system is controlled and data is processed by Thermo Electron Xcalibur™ Software. Other instruments may also be used, however optimization may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum instrument operation.

Following are the typical instrumental parameters applied for this method during method validation using a Thermo Electron TSQ Quantum Ultra mass spectrometer. The analyst should make necessary adjustments and tuning to these parameters to obtain optimum operational conditions based on the actual instrument used for the specific study.

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

4.5 Optianl LC-MS/MS Instrument (APPI) Description

HPLC System	:	Surveyor Plus LC System – A quaternary solvent system equip with MS Pump Plus
Autosampler	:	Surveyor MS Plus
Detector	:	Thermo Electron TSQ Quantum Ultra mass spectrometer with Xcalibur™ Software
Collision Gas	:	Purified Argon in compressed cylinder
Gas Supply	:	House Nitrogen supply

4.6 Optional Chromatography Conditions

Column	:	Zorbax SB-CN, 4.6 x 75 mm, 3.5 µm (Agilent Cat. no. 866953-905)
Column Oven Temperature	:	25°C
Injection volume	:	20 µL
Stop Time	:	7.0 minutes
Injection protocol	:	Analyze sequence after 4-5 warm-up injections
Sample Tray Temperature	:	20°C
Mobile phase	:	Solvent A: 0.05% formic acid in ultrapure water Solvent B: Acetonitrile

Mobile Phase Composition

Time (min)	%A	%B	Flow Rate (µL/min)
0.0	90	10	600
0.5	90	10	600
3.0	10	90	600
6.0	10	90	600
6.1	90	10	600
7.0	90	10	600

The typical retention times for the analytes are listed in Section 4.3 when using this instrumentation and conditions. The retention time may vary depending upon chromatographic conditions and systems. The chromatographic conditions employed in this method are not designed to resolve the stereoisomers in racemic mixtures.

Note: To help minimizing instrument contamination, a timed event controlled switching valve may be used to divert the LC stream to waste during periods of no data collection.

4.7 Optional Mass Spectrometer Conditions (APPI)

Ion Source Parameters (HESI-II Probe):

	APPI* Negative Mode
Spray Voltage (V)	2400
Vaporization Temperature (°C)	400
Sheath Gas Pressure (psi)	40
Ion Sweep Gas Pressure (psi)	0
Aux Gas Pressure (psi)	15
Capillary Temperature (°C)	300
Tube Lens Offset	tuned value(s)
Skimmer Offset (V)	0
Collision Pressure (mTorr)	1.0

Note: The mass spectrometer tuning parameters shown here are for reference only. The analyst should always consult with instrument operation manual to obtain optimum conditions for all the analytes prior to residue analysis.

* HPLC grade toluene is used as dopant under APPI conditions. The dopant is introduced post column with a T-joint via a syringe pump at a flow rate of 10 µL/minutes prior the LC stream entering the MS probe.

MRM (SRM) Operating Parameters:

MS/MS Transitions

Analyte	MS/MS Transition*	Scan Width	Dwell (sec.)	CE (Volts)	Q1 PW	Q3 PW	RT (min.)
Chlorothalonil	APPI Negative						
Primary	244.9 → 182.0	0.002	0.05	30	0.7	0.7	4.69
Confirmatory	246.9 → 184.0	0.002	0.05	30	0.7	0.7	4.69
Confirmatory (alternative)	244.9 → 175.0	0.002	0.05	30	0.7	0.7	4.69

APPI negative mode data collection windows: 3.0 – 5.5 minutes for chlorothalonil

4.8 Confirmatory Procedures for Chlorothalonil

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

Residues of analytes may be calculated in ppb (µg/kg) for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 20x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to the analytes of interest. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of five injections of sample solutions

c) Generate calibration curve parameters using an appropriate regression package.

d) With linear regression as an example, following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit (or "X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" (R^2) value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Alternatively, the analyte found values can be obtained directly from instrument quantification software package when appropriate regression package is applied.

f) Calculate the residue for the analyte of interest in the sample, expressed as ppb (or µg/kg), as follows:

$$\text{Residues (ppb or } \mu\text{g/kg)} = \frac{\text{Analyte found on column (pg)}}{\text{Sample injected on column (mg)}}$$

Where analyte found (pg) is calculated from the standard calibration curve and sample injected on column is calculated as follows:

$$\text{Sample injected (mg)} = \left(\frac{\text{Sample wt (g)}}{\text{Extract Vol (mL)}}\right) \times \left(\frac{\text{Aliquot vol (mL) x Inj. Vol (uL)}}{\text{Final volume (mL)}}\right)$$

Where:

$$\text{Extract Vol} = \text{extraction solvent vol (mL)} + \text{sample weight (g)} \times \text{moisture (\%)}$$

Note: The sample moisture values can be determined experimentally, following a suitable moisture content determination procedure.

If residues need to be corrected for average percentage recovery; *e.g.* for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (ppb)}$$

5.2 Single-Point Calibration Procedure

Residue of analytes may be calculated in ppb (µg/kg) for each sample using a mean standard response from each of the injections bracketing the sample as follows. Single point calibrations are generally NOT recommended for this method; particularly when analyte has non-linear detector responses.

a) Make repeated injections of a standard containing analytes of interest at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4.0. When a consistent response is obtained, measure the peak areas obtained for the analyte of interest.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to the analyte of interest.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Sometimes, residues are found in the controls due to unknown reasons. If the control peak areas are <50% LOD, no contaminations or interference should be reported. Note: Do not correct recoveries or unknown with control values if the method is used for tolerance enforcement purpose. If significant matrix effects are observed, matrix-matched standards should be used to compensate the effects or by further dilution of the final sample with 20/80 (v/v) MeOHl/0.1% formic acid in ultrapure water to reduce or eliminate these effects should instrument sensitivity permit.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade MeOH, acetone or acetonitrile prior to use.

8.0 METHOD VALIDATION

8.2 Limit of Quantification (LOQ)

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of ≤ 20% has been obtained. The LOQ of the method for chlorothalonil in soil has been confirmed to be 5 ppb (µg/kg) in the ILV study.

8.3 Limit of Detection (LOD)

The LOD of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as 4 times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The LOD was estimated to be 4 pg (or less; individual analyte) injected on column for chlorothalonil; equivalent to 0.2 pg/µL concentration when using a 20 µL injection volume with instrument setups described in the method.

8.4 Matrix Effects

As concluded in the ILV report (Reference 3), the magnitude of the matrix effects were considered not to be significant for chlorothalonil in the soil. However, matrix-matched standards may be used for calibration to compensate for any significant effects arising from matrix effects when appropriate.

8.5 Detector Linearity

For accurate quantitation of residue concentrations, analyses should be carried out within range of proper detector responses. For multi-point calibration, detector range and proper response regression (linear) shall be demonstrated within each sample set and a minimum of five different concentration levels are used for both primary and confirmatory transitions calibration plots.

In the ILV study (Reference 3), the LC-MS/MS detector responses for chlorothalonil was confirmed within the concentration range of 0.2 pg/µL to 20 pg/µL with 20 µL injections (equivalent to 4 to 400 pg standards on-column). Linear regression with 1/x weighing factor is recommended for quantitation of chlorothalonil.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested response range prior to final quantification.

8.6 Sample Final Fraction Stability

Sample final fraction in 20/80 (v/v) MeOH/0.1% formic acid in ultrapure water retained in vials and stored at a temperature of approximately 4°C were stable for at least 7 days for chlorothalonil residue analysis, [illegible]

9.0 LIMITATIONS

The method has been validated on representative soil types. It can reasonably be assumed that the method can be applied to other soil types not tested.

10.0 CONCLUSIONS

Analytical Method (GRM005.08A) is a reliable and accurate method for determination of chlorothalonil in soil, which has been validated by an ILV study (Reference 3). Only commercially available laboratory equipment and reagents are required. The analysis of 13 soil samples for chlorothalonil can be completed by one person in one day. Control and fortified control samples should be analyzed with each set of samples to demonstrate absence of any interference and adequate method performance, if possible.

The LOQ of the method is at 5 ppb (µg/kg) for chlorothalonil in soil.

This method satisfies US EPA guidelines EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

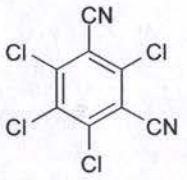
TABLE 1 Characterization Data of Soil Types used for ILV Study

Soil Type	pH (0.01 M $CaCl_2$)	Sand Content (% w/w)	Silt Content (% w/w)	Clay Content (% w/w)	Organic Carbon (%)
Clay Loam[1]	7.6	21	42	37	N/A
Sandy Loam[2]	7.3	55	28	17	N/A

CHEMICAL STRUCTURES

FIGURE 1 Chlorothalonil

Compound Code Number	:	R44686
Alternative compound code number	:	SDS2787
CAS Number	:	1897-45-6
IUPAC Name	:	2,4,5,6-Tetrachloro-isophthalonitrile
Molecular Formula	:	$C_8 Cl_4 N_2$
Molecular Weight	:	265.91
Molecular Mass	:	263.88



APPENDIX 1 Apparatus

Recommended Suppliers [Need update]

Equipment	Description	Supplier
General lab glassware	General lab glassware	www.thermoscientific.com
General lab plastic-ware	General lab plastic-ware	www.thermoscientific.com
Autosampler vials	Snap cap, 2 mL size	www.thermoscientific.com
LC-MS/MS system Includes HPLC and autosampler units	Waters Acquity UPLC system (I Class) and AB Sciex 5500 Qtrap or equivalent	www.thermoscientific.com
HPLC column	Zorbax SB-Aq, 4.6 x 50 mm, 1.8 mm	www.agilent.com
SPE Cartridges	Bond Elut C18 500 mg, 6-mL	www.agilent.com
PTFE Syringe Filter	13mm, 0.45 µm	www.thermoscientific.com

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Ultrapure water	Optima grade	www.thermoscientific.com
MeOH	HPLC grade	www.thermoscientific.com
Acetonitrile	HPLC grade	www.thermoscientific.com
Acetone	HPLC grade	www.thermoscientific.com
Formic Acid	A.C.S. grade	www.thermoscientific.com
Sulfuric Acid	A.C.S. grade	www.thermoscientific.com
Ammonium Acetate	A.C.S grade	www.thermoscientific.com
Chlorothalonil (R44686)	GLP certified	Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

a) 0.1% formic acid in ultrapure water, prepared by mixing 1 mL of formic acid in 999 mL of ultrapure water;

b) 40/60 (v/v) MeOH/0.1% formic acid in ultrapure water, prepared by mixing 200 mL of MeOH with 300 mL of 0.1% formic acid in ultrapure water;

c) 50% H_2SO_4 in ultrapure water, prepared by mixing 200 mL of ultrapure water with 200 mL of concentrated H_2SO_4;

d) Fresh extraction solvent, prepared by mixing 60 mL of 50% H_2SO_4 with 1540 mL of acetone before adding to the soil sample;

e) 50/50 (v/v) MeOH/ultrapure water, prepared by mixing 500 mL of MeOH with 500 mL of ultrapure water;

f) 20/80 (v/v) MeOH/0.1% formic acid in ultrapure water, prepared by mixing 200 mL of MeOH with 800 mL of 0.1% formic acid in ultrapure water;

g) 0.1% formic acid in ultrapure water, prepared by mixing 1 mL of formic acid with 999 mL of ultrapure water;

h) 1 M ammonium acetate aqueous solution, prepared by dissolving 7.7 grams of ammonium acetate into 100 mL of ultrapure water;

i) 0.1 mM ammonium acetate aqueous solution, prepared by mixing 100 µL of 1 M ammonium acetate solution with 1000 mL optima grade water.

APPENDIX 3 LC-MS/MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polytyrosine-1,3,6 solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for Analytes

For example, infuse a standard solution of chlorothalonil (0.1 to 1.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate at of approximately 5-20 µL/min in the presence of toluene (infused at a flow rate of 5 µL/min) as dopant under APPI conditions. Roughly adjust interface parameters (sprayer position and temperature, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 244.9 for chlorothalonil in negative ionization mode. The m/z of 244.9 represents a product ion of $[M\text{-}Cl+O]^-$ from chlorothalonil as reported in the literature.[See references below]

Using the Analyst 2 Software optimization routine, tune the instrument for chlorothalonil for MS/MS transitions and ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of standards using mobile phase at the flow rate to be used. Tune the interface parameters (sprayer position and temperature, spray and heater gas flows, spray, orifice, and focusing ring voltages and the collision gas pressure) for maximum sensitivity.

In general, the two most sensitive MS/MS transitions are selected and used for quantitative and confirmative analysis. The tuned *m/z* values and corresponding MRM transitions for the analytes are listed in Section 4.3.

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

The instrument must be mass calibrated on a regular basis using polytyrosine-1,3,6 solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

APPENDIX 4 Method Flow Chart

Weigh sample (20 g) into a Nalgene plastic bottle (250 mL)
(Fortify recovery sample, if needed)

↓

Extract with freshly made acidic acetone (100 mL) on a shaker for 2 hours

↓

Centrifuge and decant supernatant into brown bottle (125 mL)

↓

Precondition SPE cartridge (Bond Elut C18, 500 mg, 6 mL)

↓

Aliquot 2 mL of the extract and dilute with 10 mL of ultrapure water

↓

Load sample onto SPE cartridge and rinse with 3 mL of 50/50 (v/v) MeOH/ultrapure water

↓

Wash (but DO NOT dry) SPE cartridge with 3 x 4 mL of 50/50 (v/v) MeOH/ultrapure water

↓

Elute analyte from SPE cartridge with 5 mL of acetonitrile and collect

↓

Add 1 mL of 0.1% formic acid in ultrapure water

↓

Evaporate to aqueous (< 1 mL)

↓

Add 40/60 (v/v) MeOH/0.1% formic acid in ultrapure water to 2 mL

↓

Sonicate to mix well

↓

Analysis by LC-MS/MS