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Chlorothalonil

Chlorothalonil (R44686) – Independent Laboratory Validation of Analytical Method GRM005.01A for the Determination of Residues of Chlorothalonil in Crops

Final Report

DATA REQUIREMENT(S): EPA OPPTS 860.1340 (1996)
EPA OCSPP 850.6100 (2012)
SANCO/3029/99 rev.4 (2000)
SANCO/825/00 rev.8.1 (2010)

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2.0 INTRODUCTION

2.1 Purpose

The objective of this study was to independently validate analytical method GRM005.01A for measuring residues of Chlorothalonil in hybrid Bermuda grass clippings, peanut above ground foliage, potato above ground foliage, lemon and almond.

3.0 MATERIALS AND METHODS

3.1 Test Substance

Test Substance Name:	**Chlorothalonil**
CAS Number:	1897-45-6
Molecular Formula:	$C_8Cl_4N_2$
Molecular Weight:	265.9 g/mol
Sponsor Lot Number:	953519 (RJK001-206)
Purity:	99.8%
Storage Conditions:	(Room temperature) 15-30°C
Recertification Date:	End of November 2021

3.2 Experimental Design

3.2.1 Protocol Adherence

The study was performed in accordance with the protocol with three deviations and one amendment.

3.2.1.2 Amendment Details

The purpose of the amendment was as follows:

1. To modify the range that the calibration line covers of the expected nominal concentrations of quality control samples. Originally this was from 30% of the LOQ final extract concentration to 120% of the 10 × LOQ final extract concentration. The modified range was 20% of the LOQ final extract concentration to 200% of the 10 × LOQ final extract concentration. This was in response to the method modification that was required due to differences in instrument performance.
2. The addition of coefficient of determination (r^2) as a reported value.
3. Modification to the study timelines.
4. Removal of the original LOD calculation where it is estimated as four times the baseline noise. This was in response to instrument performance where no measurable baseline noise was produced for some chromatograms. Instead the LOD was calculated as the sample concentration equivalent to the lowest calibration standard.
5. After a failed validation attempt for almonds it was decided that further development would be required for high oil content matrices and that no further validation attempts would be made in this study for almonds. Therefore, the method, as it stands, is not considered suitable for high oil content matrices.

3.2.2 Analytical Standards

Analytical grade Chlorothalonil was supplied by the Sponsor. A Certificate of Analysis including purity, recertification date and lot number were provided.

All precautions required in the handling, storage and disposal of the test substances were outlined by preparation of a COSHH assessment prepared by Smithers Viscient.

The date of receipt and recertification date, plus lot number and purity details are recorded in the table below along with the allocated Test Facility (ESTS) identification number. Chlorothalonil was stored at room temperature (15 to 30°C).

Analytical Standard	ID Number	Date Received	Supplier Lot Number	Chemical Purity (%)	Recertification Date
Chlorothalonil	ESTS 148/18	24 September 2018	953519 (RJK001-206)	99.8	End of November 2021

3.2.3 Receipt and Storage of Study Samples

Control hybrid Bermuda grass clippings, peanut above ground foliage and potato above ground foliage were provided by the Sponsor. Control organic lemon and almond was purchased by Smithers Viscient from Sainsbury's in Harrogate, UK. Each control matrix was given a unique Smithers Viscient number on arrival and stored frozen (<-10.0°C, nominally -20°C).

The hybrid Bermuda grass clippings, peanut above ground foliage and potato above ground foliage were already processed prior to shipment by the Sponsor (and the acid for stabilisation had already been added to the potato above ground foliage). Ground almonds were purchased, so required no further processing. The whole lemons were homogenised in the presence of dry ice according to local SOPs.

3.2.4 Analytical Method

Analytical method GRM005.01A was supplied by the Sponsor. The method employed GC-MSD with chemical ionisation.

3.2.4.1 Summary of the Analytical Method GRM005.01A

The method GRM005.01A provided by the study Sponsor was independently validated at Smithers Viscient (ESG) Ltd. under the analytical procedure SMV3202297-01D.

The method involved acidifying aliquots of untreated control hybrid Bermuda grass clippings, peanut above ground foliage and lemon (potato above ground foliage had already been acidified during processing; almond is considered a dry commodity and so did not require acidification) before fortifying each matrix with known amounts of Chlorothalonil. The fortified samples were then extracted, using maceration with an ultra-turrax homogeniser, with an amount of acidified acetone dependent on water content of the commodity. Water content information was derived from data previously collected by the Sponsor, online sources (USDA National Nutrient Database for Standard Reference, Release 1 April 2018 found at https://ndb.nal.usda.gov/ndb/ and by determination at Smithers Viscient. The samples were then subjected to SPE clean-up before dilution and injection on the GC-MS.

The recoveries of Chlorothalonil for each sample in each matrix were determined.

It is estimated that the total person hours required to analyse one set of samples from the start of extraction to completion of instrumental analysis (data processing) is 7.5 hours.

3.2.4.2 Linearity

In order to establish linearity of response of the analytical chromatographic system to Chlorothalonil, eight mixed standard solutions of increasing concentration were prepared in both toluene and untreated control matrix over the range 0.0001 to 0.01 µg/mL. The lowest concentration was equivalent to the concentration of a sample extract at 20% of the limit of quantification (LOQ) and the highest concentration was equivalent to the concentration of a sample extract at 200% the LOQ. Matrix matched calibration standards were injected into the chromatograph in random order interspersing the samples. Non-matrix matched calibration standards were injected in the same order as the matrix matched standards but after all the matrix samples to ensure no interference of the solvent to the chromatography. Concentration/response curves were prepared and matrix effects were calculated.

3.2.4.3 Specificity / Selectivity

The ability of the method to distinguish between Chlorothalonil from other substances present in the control samples was investigated. Components present in a blank sample that interfered with the analysis at levels ≥ 30% LOQ would have been deemed significant.

3.2.4.4 Precision

Repeatability of the method was demonstrated by analysing each fortified validation level in quintuplicate. The RSD at each validation level was determined and considered acceptable if ≤ 20%. Confidence intervals were also calculated.

3.2.4.6 Limit of Quantification (LOQ)

The limit of quantification was defined as the lowest fortification level where an acceptable mean recovery (70 to 110%) for Chlorothalonil was obtained and a relative standard deviation of ≤ 20% was achieved. For this analytical method, the limit of quantification was proposed as 0.01 mg/kg for Chlorothalonil in hybrid Bermuda grass clippings, peanut above ground foliage, potato above ground foliage, lemon and almond.

3.2.4.7 Limit of Detection (LOD)

The LOD was calculated as the sample concentration equivalent to the lowest calibration standard.

3.2.4.8 Matrix Effect Assessment

The effect of matrix on Chlorothalonil was assessed by comparison of peak area between solvent standards and equivalent standards prepared using control matrix.

Matrix effects were considered significant if the difference between the peak areas of these standards was ≥ 20%.

3.2.4.9 Final Extract Storage Stability

The stability of the final extracts were assessed under refrigerated conditions (2-8°C) after a minimum period of seven days storage. Each set of vials injected for the initial validation were stored and re-injected after the storage interval. Both matrix and non-matrix standards were re-injected.

The final extracts were considered stable if all mean recovery, precision, specificity and linearity criteria were achieved. Final extract storage stability re-injection was only performed if the initial validation injection passed acceptance criteria.

4.1.1.6 Limit of Quantification (LOQ)

Acceptable accuracy and precision of recovery (based on guideline criteria) at the lower validation level confirmed the limit of quantification (LOQ) of the method as 0.01 mg/kg for Chlorothalonil in hybrid Bermuda grass clippings, peanut above ground foliage, potato above ground foliage and lemon.

4.1.1.7 Limit of Detection (LOD)

The Limit of Detection (LOD) for the analytical method was calculated to be 0.002 mg/kg for all ions and all matrices.

APPENDIX 3 Water Content

Matrix	Water Content (%)	Reference
Hybrid Bermuda Grass Clippings	66.4	LNB 7549/29-Where moisture was calculated-RSTS06803, INV20134.1
Peanut Above Ground Foliage	78.16	3202297 Narr-3
Potato Above Ground Foliage	88.0	LNB 7370/81 Where moisture was calculated RISP01202, INV19597.1
Lemon	87.4	FDA PAM Volume 1 Section 201
Almond	4.41	USDA National Nutrient Database for Standard Reference, Release 1 April 2018 found at https:// ndb.nal.usda.gov/ndb/

Analytical Procedure

Procedure Title	Chlorothanonil (R44686): Analytical Method for the Determination of Residues in Whole Lemons, Above Ground Potato Foliage, Above Ground Peanut Foliage and Bermuda Grass Clippings

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1. INTRODUCTION AND SUMMARY

The analytical procedure described is suitable for the determination of residues of Chlorothalonil in whole lemons, above ground potato foliage, above ground peanut foliage and Bermuda grass clippings using an external standardisation procedure. The limit of quantification (LOQ) is 0.01 mg/kg for all matrices. This document is based on Syngenta analytical procedure GRM005.01A.

1.1. Method Summary

Crop samples are extracted by homogenisation with acetone:5 M sulphuric acid solution (95:5 v/v) and then centrifuged. Aliquots are diluted with water followed by a solid phase extraction (SPE) clean-up. Chlorothalonil is analysed by gas chromatography with mass selective detection (GC/MS).

1.2. Equipment and Apparatus

The recommended equipment is described in Appendix 1. Equipment with equivalent performance specifications can be substituted provided that they can be shown to be suitable.

1.3. Reagents

Particular care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method. Alternative reagents of comparable purity can be used.

1.4. Preparation of Analytical Standard

Analytical standards of Chlorothalonil should be prepared and stored in acid washed amber volumetric flasks. The amber glassware prevents potential photolytic degradation of Chlorothalonil. The acid washing removes any detergent residues present, which may cause instability problems.

Glassware should be soaked for a minimum of one hour in 1M hydrochloric acid solution, and then rinsed in ultra-pure water followed by acetone and a further rinse with toluene. Finally the glassware should be left to dry before use.

Weigh out accurately, using a five figure balance, sufficient Chlorothalonil analytical standard to allow dilution in toluene to give a 1000 µg/mL stock solution in a volumetric flask. The Chlorothalonil stock solution is then serially diluted in toluene to 0.01 µg/mL. These standards may be used for fortification of recovery samples prior to extraction and for GC/MS final determination. It is recommended that a 1000 µg/mL stock solution is prepared for Chlorothalonil. This allows the use of small standard volumes for high fortification levels to minimise any immiscibility issues.

When not in use, always store the standard solutions in a refrigerator (2 - 8°C) to prevent decomposition and/or concentration of the standard.
Chlorothalonil analytical standards should be replaced with freshly prepared standards after six months.

1.5. Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure.

Acceptable external standard recoveries will validate the work stoppages. Samples should be stored refrigerated (2 - 8°C) in sealed vessels.

2. PROCEDURES

All procedures will be carried out in compliance with departmental SOPs.

2.1. Sample Preparation

2.1.1. Validation Samples

For validation purposes, the commodities used are untreated with Chlorothalonil, therefore, instability is not a concern until after the samples have been fortified. If control commodities have already been processed without the presence of the 1 M H_2SO_4, it is acceptable to add 10 mL of the acid per 100 g of the (already homogenised) crop and then mixing just prior to weighing for analysis.

2.2. Extraction

a) Weigh representative amounts of crop matrix equivalent to 10 g (i.e. 11 g for wet crops prepared with acid) into screw-capped plastic bottles (approximately 250 mL size).

b) For quality control or validation purposes, fortify samples of untreated control with known amounts of Chlorothalonil in toluene (not more than 0.5 mL fortification volume).

c) Add acetone:5 M sulphuric acid solution (95:5 v/v, 100 mL minus the water content of the samples, and minus a further 1 mL for crops prepared with acid).

Note: Estimate the percentage water content in each matrix type and hence the total volume of water in the 10 g sub-sample. E.g. for a 10 g sub-sample with 90% natural water content add 100 mL — (10 x 90/100) mL = 91 mL extraction solution. It is sufficient to round the natural water content to the nearest ten percent value. Any volume contraction from mixing organic solvents with water and evaporation loss during extraction is considered to be negligible.

d) Homogenise at high speed for 3 - 5 minutes using an Ultra-Turrax homogeniser. The use of detergent for cleaning should be avoided as any residues remaining on glassware may cause instability problems with Chlorothalonil.

e) Centrifuge samples at 3500 rpm (or at a speed that visibly separates the solid sample from the supernatant) for approximately 5 minutes.

2.3. Sample Clean-up

a) Transfer aliquots of the crop extracts equivalent to 0.2 g into a disposable glass test tube (10 mL size). Add water (6 mL) to the aliquots and mix.

b) Take one C8 (EC) solid phase extraction cartridge (size 100 mg, 3 mL) for each sample to be analysed and place on a suitable vacuum manifold. Add methanol (3 mL) and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add water (3 mL) to the top of each cartridge and draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluates. Do not allow the cartridges to become dry.

c) Transfer the sample aliquots from section 2.3 (a) onto the prepared columns and allow to percolate onto the cartridges under gravity or low vacuum (approximately 200 mbar). Discard the column eluates.

d) Add water: acetonitrile (70:30, 1.5 mL) to the top of each cartridge and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL/min. Discard the wash. Remove any remaining droplets of water adhering to the insde of the cartridge with absorbent tissue and dry the cartridges under high vacuum (>500 mbar) for approximately 15-20 minutes.

   Note: Where achievable vacuums are less than specified or apparatus does not allow sufficient air flow through the cartridges, longer drying times may be required.

e) When the cartridges are dry, place graduated polypropylene collection tubes (15 mL) under each port, as required, in the manifold rack. Add toluene (2 mL) to the top of each cartridge to elute Chlorothalonil, and draw through under vacuum at a rate of approximately 2 mL/min, removing the excess solvent from the column.

   Note: Prior to using the test tubes perform a visual check on a random selection of three tubes. Pipette toluene (4 mL) into the tube to confirm that the solvent meniscus corresponds to the 4 mL graduation mark on the tube.

f) Adjust the final volume to 4 mL with toluene. Sample concentration is now 0.05 g/mL. Transfer 1 mL aliquots from each sample into GC auto-sampler vials. Cap and crimp the vials and analyse for chlorothalonil by GC-MS using negative-ion chemical ionization.

g) During validation for an assessment of the degree of matrix effects, inject (within the same validation run) a set of equivalent calibration standards in toluene without the presence of matrix alongside the matrix matched calibration.

   The preparation of matrix matched calibration standards is described in Section 2.4.

   Note: The above SPE procedure has been developed using columns from the stated manufacturer, however, it is possible to carry out the procedure using similar columns from other manufacturers. In all cases, it is strongly recommended that the elution profile of the chosen batch of columns is checked prior to commencing analysis. This will eliminate any variation between manufacturers' products and between batches.

2.4. Matrix Matched Calibration Standards

Matrix-matched calibration standards may be prepared from analytical standards prepared in Section 1.4, as follows:

For example, to prepare a 0.001 µg/mL Chlorothalonil matrix-matched standard, take 0.99 mL of the control sample in toluene from Section 2.3 (f) and place in a suitable auto-sampler vial. Add 10 µL of a 0.1 µg/mL standard in toluene. Ultrasonicate and vortex mix thoroughly. The concentration of the matrix-matched calibration standard is now 0.001 µg/mL. For the GC/MS used in this procedure, a calibration range of 0.0001 to 0.01 µg/mL was employed. An alternative calibration range can be employed depending on the instrument performance providing all sample responses are within this calibration range.

2.5. Final Extract Stability

It has been shown that final extracts are stable (when stored refrigerated) for up to 7 days after completion of the extraction.

2.6. Chlorothalonil Final Determination by GC-MS

Instrumentation:	Thermo TSQ8000 Evo GC-MS/MS Used in single MS (SIM) mode	
Column#:	SGE BPX-50, 15 m x 0.25 mm, 0.25 µm film	
Carrier gas (flow):	Helium (1.0 mL/min constant flow and vacuum compensation)	
MS mode#:	Negative chemical ionisation with methane reagent gas at 1 mL/min (CI-)	
Electron energy	70 eV	
SSL injection port liner#:	Carbofrit™ insert in 4 mm ID base deactivated gooseneck liner	
Injection volume:	1 µL, splitless with surge	
Surge pressure and duration:	200 kPa for 1 min	
Septum purge flow:	5 mL/min	
Septum purge stop duration:	2 minutes	
Needle wash program:	2 pre and post injection washes with toluene	
Column temperature program:	120°C initial, hold for 1 min. Ramp at 20°C/minute to 300°C	
Transfer line temperature:	280°C	
Ion source:	150°C	
SIM Ions monitored#	Time	*m/z*
	4 min to end of run	266, 264 and 268
Dwell time	0.2	
Expected retention time (approximate):	Chlorothalonil: 5.9 minutes	

3. CALCULATION OF RESULTS

All peak measurements and calculations are performed on Chromeleon 7.2 SR4. From the measured peak area, where the calibration fit is linear as in this study, Chromeleon uses the following formula to calculate the concentration of test substance present in the sample.

$$A\ (mg/kg) = \frac{\text{Peak Area - Constant}}{1^{st}\ \text{Degree}} \times \frac{UP3 \times UP2}{UP1}$$

Where:-

A (mg/kg)	= Amount of Chlorothalonil
Peak Area	= Peak area ratio due to Chlorothalonil
Constant	= Y intercept on calibration graph
1st Degree	= Slope of calibration graph
UP1	= Sample weight (g)
UP2	= Final volume (mL)
UP3	= Dilution factor

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery}\quad (\%) = \frac{A - C}{S} \times 100$$

Where:-

A = concentration found in fortified sample (mg/kg)
C = concentration (or interference) found in control sample (mg/kg)
S = concentration added to fortified sample (mg/kg)

APPENDIX 1

List of Apparatus/Equipment and Suppliers

- Trace 1300 Gas Chromatograph, Thermo
- Triplus RHS auto-sampler, Thermo
- TSQ8000 MS/MS, Thermo (used in single MS mode)
- Ultra Turrax T25 (18 mm diameter head) homogeniser motor and disperser, ThermoFisher UK
- Centrifuge Beckman Coulter, Allegra X15R
- Dri-Block sample evaporator, Büchi
- GC Column BPX-50, 15 m x 0.25 mm, 0.25 µm film, SGE
- GC liner Carbofrit™ insert in 4 mm ID base deactivated gooseneck liner, Thames Restek
- Isolute, C8(EC), 100 mg, 3 mL Solid Phase Extraction Cartridges, Biotage
- VacMaster™ 20 vacuum manifold, Biotage
- Various positive displacement volumetric pipettes (Gilson, Rainin etc.)
- Plastic pots various volumes, Technical Treatments UK
- 15 mL Graduated polypropylene centrifuge tubes, Fisher UK
- 1.5 mL Borosilicate glass injection vials (with lids), Agilent

APPENDIX 2

List of Chemicals and Suppliers

- LC-MS Grade water, In-house Milli-Q
- ≥99.8% HiPerSolv Toluene, VWR
- ≥99.8% HiPerSolv Acetone, VWR
- ≥99.9% HiPerSolv Acetonitrile, VWR
- ≥99.8% HiPerSolv Methanol, VWR
- ≥97.5% Sulphuric acid, Fluka
- 1 M Hydrochloric acid, Fluka
- BIP® grade Helium carrier gas, Air Products UK
- PR 4.5 grade Methane reagent gas, Air Products UK

Reagents and Preparation Method

- 5M Sulphuric acid
  Slowly add 273.37 mL of sulphuric acid (≥97.5%) to 726.63 mL of water
- 1M Sulphuric acid
  Slowly add 50 mL of 5M sulphuric acid to 200 mL of water
- Acetone: 5M sulphuric acid (95:5 v/v)
  Mix 950 mL of acetone and 50 mL of 5M sulphuric acid
- Water: acetonitrile (70:30 v/v)
  Mix 700 mL of water with 300 mL of acetonitrile

APPENDIX 7 Schematic Diagram of Analytical Method