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Chlorothalonil

Chlorothalonil - Independent Laboratory Validation of Analytical Method (GRM005.13A) for the Determination of Chlorothalonil in OVS Silica Gel Air Sampling Tubes by GC-NICI-MS

Final ILV Report

DATA REQUIREMENT(S): EPA 850.6100

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VOLUME 1 OF 1 OF STUDY

2.0 INTRODUCTION

Described in this report is the independent laboratory validation of Syngenta Analytical Method GRM005.13A (Reference 1) as performed by PASC.

This study was designed to satisfy guideline requirements described in EPA 850.6100 (2012) (Reference 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The residue analytical method is deemed suitable for the determination of Chlorothalonil in OVS Silica Gel Air Sampling Tubes.

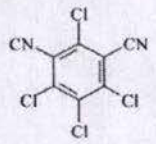
OVS (520/260) air sample tube contents are extracted with 10 mL acetone. An aliquot of sample is evaporated to dryness and reconstituted with an appropriate amount of toluene and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for analysis.

The validated limit of quantitation of method GRM005.13A is 0.01 µg/OVS (520 mg/260 mg) air sampling tube.

3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

The test/reference substance was obtained from Syngenta Crop Protection, LLC. The following test/reference substance was used:

Compound Structure	
Syngenta Code:	R44686
Common Name:	Chlorothalonil
CAS Name:	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
Batch ID:	638406
Molecular Weight:	265.91 g/mol
Storage Conditions:	Refrigerate < 30°C
Purity:	99.6%±0.5%
Expiration Date:	01/2018

Characterization data for the test/reference standard are maintained by Syngenta Crop Protection, LLC. The Certificate of Analysis is included in Appendix 2.

The test/reference substance (Chlorothalonil) used in this study was procured from Syngenta Crop Protection, LLC located at the Greensboro facility. All solutions made from chlorothalonil standard were stored according to Section 2 of the method.

3.2 Test System

The test system evaluated for this ILV was OVS Silica Gel Air Sampling Tubes (SKC Inc. Cat No. 226-99, Lot#9251).

3.3 Equipment and Reagents

The equipment and reagents used for the ILV were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. All solvents and other reagents must be of high purity, e. g. glass distilled/HPLC grade solvents and analytical grade reagents.

3.4 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in Section 2 of the method (Reference 1).

3.4.1 Stock Standard

One 101 µg/mL stock solution for chlorothalonil was prepared in toluene.

3.4.2 Fortification Standard

Sample fortification solutions containing chlorothalonil were prepared by serial dilution in toluene from the stock solution. The following solutions were prepared: 10 µg/mL and 1 µg/mL for fortification purposes.

3.4.3 Calibration Standard

Calibration standards were prepared by serially diluting stock standards using toluene. Using equivalent GC-MS instrumentation described in the method, the following concentration range of standards were prepared and used to construct the calibration plots for *m/z* ions 266, 264, and 268 (0.10 µg/L – 50.72 µg/L).

3.5 Analytical Procedures and Modifications

Analytical Method GRM005.13A (Reference 1) was successfully validated by an independent laboratory as written using the procedures and instrumentation recommended by the method. OVS (520/260) air sample tube contents are extracted with 10 mL acetone. An aliquot of sample is evaporated to dryness and reconstituted with an appropriate amount of toluene and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for mz/ 266,

264, and 268 for analysis. The limit of quantitation of Analytical Method GRM005.13A (Reference 1) is 0.01 µg/OVS (520 mg/260 mg) air sampling tube.

3.5.1 Modifications

Syngenta Analytical Method GRM005.13A (Reference 1) was followed as written.

3.5.2 Fortifications

Untreated control OVS Silica Gel Air Sampling Tubes samples were fortified using 10 µL of known amounts of chlorothalonil to LOQ and 10X LOQ concentration levels as per the method. See Table 2 for detailed fortification levels. Fortifications used in this ILV are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/mL)	Replicates
LOQ	10	1.01	5
10X LOQ	10	10.14	5

3.5.3 Method Summary

As per Analytical Method GRM005.13A, OVS (520/260) air sample tube contents are extracted with 10 mL acetone. An aliquot of sample is evaporated to dryness and reconstituted with an appropriate amount of toluene and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for mz/ 266, 264, and 268 for analysis.

3.5.4 Limit of Detection and Limit of Quantitation

The limit of detection (LOD) of the method is defined as the lowest analyte amount injected on column detectable above the mean amplitude of the background noise at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. The limit of detection using the instrumentation for this validation was estimated to be 0.1 pg/µL. Note that the LOD may vary between runs and from instrument to instrument.

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated with a mean recovery of 70 - 120% and a relative standard deviation of $\leq$ 20% has been obtained. A limit of quantitation (LOQ) of 0.01 µg/OVS Silica Gel Air Sampling Tubes was successfully validated in this study.

3.5.5 Detector Linearity

The linearity of the detector response was assessed using a calibration curve generated with each analysis sequence injected. It was shown that the GC-MS detector response for chlorothalonil has a correlation coefficient greater than 0.99 in the range from 0.10 pg/uL to 50 pg/uL or 0.20 pg to 100 pg on column when using a 2 µL injection volume.

Representative plots of the detector responses versus the analyte concentration for all calibration points are presented in the Figures Section.

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by "Chemstation Software version G1732BA, B.02.00.589". A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of the correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations, and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using Microsoft Office Excel (2010).

TABLE 2 Fortification Levels

Matrix	Fortification Level (µg/tube)	Number of Replicates
OVS Silica Gel Air Sampling Tubes	Control	2
	0.01	5
	0.1	5

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical Method GRM005.13A is suitable for the determination of chlorothalonil (Figure 1) in OVS silica gel sorbent (520 mg/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99). The limit of quantitation (LOQ) of the method has been established at 0.01 µg/OVS (520/260) air sampling tube.

This method satisfies US EPA 850.6100 guidelines.

1.2 Method Summary

OVS (520/260) air sample tube contents are extracted with 10 mL acetone. An aliquot of sample is evaporated to dryness and reconstituted with an appropriate amount of toluene and submitted to negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for analysis.

The limit of quantitation of the method is 0.01 µg/air sample type.

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 a 100 µg/mL stock solution for chlorothalonil by one of the following methods: Note: optional C6 chlorothalonil internal standard may also be prepared if matrix effect is observed to be > 20%.

Weigh out accurately, using a five figure balance, sufficient chlorothalonil analytical standard into an amber "Class A" volumetric flask (100-mL). Dilute to the mark with toluene and mix well to give a 100 µg/mL stock solution of chlorothalonil. Standards should be prepared in amber bottles and stored under refrigeration.

Alternatively, the appropriate volume of solvent added 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 solvent 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 should be prepared by serial dilution with toluene. It is recommended that the following solutions are prepared: 10 µg/mL, 1.0 µg/mL and 0.10 µg/mL for fortification purposes. A 0.10 µg/mL (100 ppb) C6 chlorothalonil standard should be prepared for internal standard, if required using toluene.

2.3.3 Preparation of Calibration Standards

No significant matrix effects, suppression or enhancement of the instrument response has been observed in the filter types tested using the procedures described in Section 3 during method development and non-matrix matched calibration standards should normally be used for quantitation using GC-NICI-MS.

A calibration curve should be generated to quantify chlorothalonil. Standards over an appropriate concentration range should be prepared with a minimum of five levels using the recommended standard range 0.1µg/L - 50µg/L in toluene (0.2 pg to 100 pg on column using a 2µL injection).

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in amber bottles and refrigerated 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 six months for chlorothalonil 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

	Acetone	Toluene
Harmful Vapor	✓	✓
Highly Flammable	✓	✓
Harmful by Skin Absorption	✓	✓
Irritant to respiratory system and eyes	✓	✓
Causes severe burns	*	*
OES Short Term (mg/m^3)	3560	560
OES Long Term (mg/m^3)	1780	188

N/A not known

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 as shown in Appendix 3. In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included in each sample set. At least one untreated control and two control samples fortified with known amounts of chlorothalonil should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

3.1 Sample Preparation

All samples should be prepared/collected using an approved method of preparation to minimize any possible cross-contamination from sample to sample. Evaluation of potential compound break-through should be determined using the specific air filter type identified in the study. Tandem configuration may be required depending on the expected residue level. No break-through was observed using OVS silica gel (520/260) air sampling tube (SKC Inc. Cat. No. 226-99) for a period of 24 hours at an in-flow rate of 1Liter/minute.

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 air sample, add the appropriate amount of standard solution (10µL recommended) containing chlorothalonil in toluene. Let each sample stand for at least five minutes after fortification to allow the spiking solvent to evaporate 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

1. Remove all contents (media, filter, foam plug, plastic holder…etc.) of air sample by pushing forward from small opening to large opening with a glass rod or suitable implement directly into appropriately size vessel (15mL polypropylene tube). To avoid contamination from the exterior surface of the air sampler, the glass OVS tube should not be included in the extraction.
2. Extract sample with 10 mL acetone by shaking for 2-3 minutes ensuring the contents are being agitated.
3. Aliquot 1.0 mL of extract and transfer into a 15 mL polypropylene tube and evaporate to dryness under a gentle stream of nitrogen or air with temperature setting of ≤ 40°C.
4. Reconstitute sample with 1.0 mL toluene, vortex to mix.
5. Further dilutions with toluene may be made based on expected residue.
6. Transfer final fraction to a GC autosampler vial and analyze by GC-NICI-MS.

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 25 samples. One skilled analyst can complete the analysis of 25 samples in 1 day (8 hour working period).

3.5 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends 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.

3.4 Problems and Modifications

If matrix effect is present, the use of internal standard is recommended.

Proper lab techniques should be implemented to prevent possible contamination of samples, labware and instrumentation.

4.0 FINAL DETERMINATION

4.1 Instrument Description (GC-NICI-MS)

GC System	:	Hewlett Packard 6890
Detector	:	Hewlett Packard 5973

4.2 Chromatography Conditions (GC-NICI-MS)

Column : HP-5MS (30.0m x 0.25 mm i.d)

Injection Port : Split/Splitless operated in splitless mode

Liner : Carbofrit Gooseneck 4 mm i.d.

Carrier Gas : Helium at 1.0 mL/min

Injection Mode : Pulsed (pressure 30 psi)

Purge Time : 2 minutes

Injection Volume : 2 µL

Injector Temperature : 250°C

Transfer Line Temperature : 280°C

Ion Source Temperature : 230°C

Quadrupole Temperature : 150°C

Oven Temperature Gradient

Step	Rate (°C/min)	Temperature	Time (min)
1	-	120	1
2	20	300	2

Under these conditions the retention time for chlorothalonil is approximately: 7.1 min.

4.3 Mass Spectrometer Conditions (GC-NICI-MS)

Chlorothalonil:

Ionization Mode	:	Chemical
Polarity	:	Negative
Calibration	:	AutoTune
Analyte	:	Chlorothalonil
Target Ion	:	266 *m/z*
Qualifier 1	:	264 *m/z*
Qualifier 2	:	268 *m/z*
Ion Ratio	:	100:65:55

Representative chromatograms are shown in the Figures Section.

4.4 Confirmatory Procedures for Chlorothalonil

Final determination by GC-NICI-MS with two qualifier ions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi Point Calibration Procedure

Chlorothalonil may be calculated in µg/sample for each sample as follows:

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 30% LOQ to 20 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five levels). And combined with an internal standard to normalize data.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to respective target ions. Quality Control standard solutions should be interspersed throughout the analysis to monitor any matrix effects.

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

d) The 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 (slope) of the line of best fit ("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" value for the regression.

Re-arrangement for x gives

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

e) Calculate residues of interest in a sample, expressed as µg/sample, as follows:

$$\text{Residue (ug/sample)} = \frac{\text{Analyte found } (\mu g/\text{mL})}{\text{Sample Vol. (mL)}}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample vol. is the final sample dilution in mL.

f) Determine the recovery by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery as a percentage (%) by the equation:

$$Recovery\ (\%) = \frac{(Residue\ in\ Recovery\ Sample) - (Residue\ in\ Control)}{Amount\ Fortified} \times 100\%$$

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

$$Corrected\ Residue = \frac{Residue\ x\ 100}{Average\ percentage\ Recovery}\ (mg/kg)$$

5.2 Single Point Calibration Procedure

Chlorothalonil may be calculated in µg/sample for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing chlorothalonil at an appropriate concentration operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for chlorothalonil.

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

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

d) Calculate the chlorothalonil residues in the sample, expressed as µg/sample using a mean standard response from each of the injections bracketing the sample as follows:

$$\text{Residue (ug/sample)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Vol.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample volume (mL)

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

$$\textbf{\textit{Corrected Residue}} = \frac{\textbf{\textit{Residue x 100}}}{\textbf{\textit{Average percentage Recovery}}} \textbf{\textit{(mg/kg)}}$$

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analyzed with each batch of samples. Control samples from the same matrix are recommended to monitor any instrumental matrix effects present.

At least two recovery samples (control samples accurately fortified with known amounts of analyte), including one at the method LOQ and one at the expected residue level, should also be analyzed alongside each set of samples. The fortification levels should be appropriate to the residue levels expected in the sample.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of ≤ 20%.

When the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix

GC-NICI-MS is a highly specific detection technique. Interferences arising from the matrices tested have not been observed.

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 methanol, acetone or acetonitrile prior to use.

FIGURE 1 **Chemical Structure**

Common Name : Chlorothalonil

Code Name : SDS2787

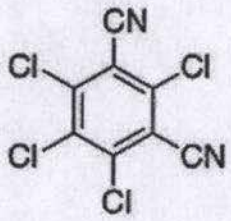
CA Index Name : 1897-45-6

IUPAC : 2,4,5,6-Tetrachloro-isophthalonitrile

Molecular Formula : $C_8Cl_4N_2$

Molecular Weight : 265.91

Molecular Mass : 263.88



APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General lab glassware	General lab glassware	www.thermoscientific.com
General lab plastic-ware	General lab plastic-ware	www.thermoscientific.com
GC Column	HP5-MS, 30m x0.25 m, x0.25 µm	www.agilent.com

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Acetone	HPLC grade	www.thermoscientific.com
Toluene	HPLC grade	www.thermoscientific.com
Chlorothalonil analytical standards	GLP certified	Syngenta Crop Protection, LLC

APPENDIX 3 Method Flow Chart

Transfer contents of OVS sampler into a 15 mL poly tube
↓
Add 10 mL acetone and agitate for 2-3 minutes
↓
Transfer 1 mL into a 15 mL poly tube and evaporate to dryness
↓
Reconstitute sample with toluene
↓
Transfer to vial and submit for GC-NICI-MS analysis