

2.0 INTRODUCTION

Described in this report is the independent laboratory validation of Syngenta Residue Method GRM043.05B (Reference 1) as performed by PASC.

This study was designed to satisfy guideline requirements described in EPA 850.6100 , EU SANCO/3029/99 Rev.4 and EU SANCO/825/00 Rev.8.1. This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

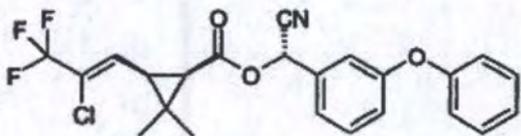
The residue analytical method is deemed suitable for the determination of lambda-cyhalothrin in soil.

10 g sub samples of soil are extracted with acetonitrile. The extracts are cleaned using n-hexane liquid-liquid partition followed by florisil SPE procedure. The final extracts are evaporated to dryness and dissolved in toluene. Final determination is by gas chromatography with mass selective (GC-MS) detection using negative ion chemical ionization.

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: | CGA 337745 |
| Common Name: | Lambda-cyhalothrin |
| Batch Identification: | DAH-XXXV-95 |
| Storage Conditions: | Refrigerate < 10°C |
| Purity: | 97.2% |
| Expiration Date: | 02/2017 |

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 (lambda-cyhalothrin) used in this study was procured from Syngenta Crop Protection, LLC located at the Greensboro facility. All solutions made from lambda-cyhalothrin standard were stored according to Section 2 of the method.

3.2 Test System

The test system evaluated for this ILV was Clay Loam Soil (CAPY081512) and Sandy Loam Soil (EXCEL FARM). This matrix was chosen because it is representative of the matrices the method was designed for. The control samples used in this study were provided by Syngenta Crop Protection, LLC. These control soil samples were characterized by AGVISE Laboratories of Northwood, North Dakota. GLP characterization results in more detail are presented in Table 1 and summarized below:

| USDA Textural Class | Sample ID | pH | Calcium (ppm) | Magnesium (ppm) | Sodium (ppm) | Potassium (ppm) | Hydrogen |
|---------------------|------------|-----|---------------|-----------------|--------------|-----------------|----------|
| Clay Loam | CAPY081512 | 6.9 | 1860 | 1080 | 47 | 272 | 29 |
| Sandy Loam | EXCEL FARM | 7.8 | 1191 | 233 | 48 | 142 | 11 |

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 100 µg/mL stock solution for lambda-cyhalothrin was prepared in toluene.

3.4.2 Fortification Standard

Sample fortification solutions containing lambda-cyhalothrin were prepared by serial dilution in toluene from the stock solution. The following solutions were prepared: 0.1 µg/mL and 1.0 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 241, 243, and 205 (0.2 µg/L - 10 µg/L).

3.5 Analytical Procedures and Modifications

Analytical Method GRM043.05B (Reference 1) was successfully validated by an independent laboratory as written using the procedures and instrumentation recommended by the method. A 10 g sample of soil is extracted using 40 mL of acetonitrile for 30 minutes by refluxing. The extracts are cleaned using n-hexane liquid-liquid partition followed by florisil SPE procedure. The final extracts were evaporated to dryness and dissolved in toluene. Final determination is by gas chromatography with mass selective (GC-MS) detection using negative ion chemical ionization.

3.5.1 Modifications

Syngenta Analytical Method GRM043.05B (Reference 1) was followed as written. No modifications were made.

3.5.2 Fortifications

Untreated control soil samples were fortified using 0.1 mL of known amounts of Lambda-cyhalothrin 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 Level | Fortification Volume (mL) | Fortification Conc. ($\mu\text{g/mL}$) | Final Volume (mL) | Replicates |
|------------|---------------------|---------------------------|--|-------------------|------------|
| Clay Loam | LOQ | 0.1 | 0.1 | 1 | 5 |
| Sandy Loam | 10X LOQ | 0.1 | 1.0 | 1 | 5 |

3.5.3 Method Summary

As per Analytical Method GRM043.05B, a 10 g sample of soil is extracted using 40 mL of acetonitrile for 30 minutes by refluxing. The extract is cleaned using an n-hexane liquid-liquid partition followed by a florisil SPE procedure. The final extract is evaporated to dryness and dissolved in toluene. Final determination is by gas chromatography with mass selective (GC-MS) detection using negative ion chemical ionization mode for (m/z) ions 241, 243, and 205.

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 the background noise. The LOD using the instrumentation for this validation was estimated as 0.8 pg injected on column, equivalent to 0.0002 $\mu\text{g/mL}$ when using a 4 μL injection volume. Note that the LOD may vary between runs and from instrument to instrument.

STUDY OBJECTIVE

Syngenta Analytical Method GRM043.05B (Reference 1) was designed/developed for residue determination of Lambda-cyhalothrin in soil. The limit of quantitation (LOQ) of the method has been proposed at 0.001 mg/kg (1 ppb) for analysis of Lambda-cyhalothrin in soil.

The objectives of this independent laboratory validation (ILV) study are:

- (1) To provide validation data, i.e. recovery rates (accuracy) and relative standard deviation (precision) per the analytical procedures outlined in the method.
- (2) To demonstrate the linearity of calibration curves.

The current guidance documents for this study are available in the Reference Section.

REFERENCE STANDARDS

Reference standard CGA337745 will be supplied by Syngenta Crop Protection, LLC. A Material Safety Data Sheet (MSDS) should accompany the reference standard, if available. The relevant information on the standard is summarized as follows:

| STANDARD | Batch ID | Synonyms | PURITY (%) | EXPIRATION DATE | STORAGE |
|--------------------------------|-------------|------------|------------|-------------------|---------|
| Lambda-cyhalothrin (CGA337745) | DAH-XXXV-95 | CSAA421914 | 97.2 | February 28, 2017 | 2-8 °C |

Source: Syngenta Crop Protection, LLC

MATERIALS/EQUIPMENT

In this study, a GC/MSD system (GC6890 + MSD5973) will be used for the determination of residues in sample final fractions with one target and two qualifier ions monitored as specified in the method. The sources and grades of the solvents and chemical reagents will be selected per the requirements from the method. If replacements have to be made, the sources, grades and part numbers will be documented in the study records.

TEST SYSTEM

The test system for this study will be soil. The untreated control sample (UTC) will be provided by the Study Sponsor, along with the characterization information.

| SAMPLE | MATRIX | SAMPLE DESCRIPTION |
|------------|--------|--------------------|
| PASC ID: * | Soil | * |

*The soil sample PASC ID, description and characterization will be included in the report.

The soil sample will be stored frozen after arrival at PASC, and will be taken out prior to analysis.

EXPERIMENTAL DESIGN

Prior to conducting sample analysis, PASC will establish method control not limited to but including analyte retention time, linearity, instrument response, instrument detection limits, procedures, and verification that the control soil matrix is free of interferences. PASC will demonstrate method control by performing assessment tests before proceeding to method validation trials. More than one assessment test may be made depending on the number and type of substitutions. Data and results of any assessment test shall be included in the study records, but not in the final report.

Clarification or interpretation of the method will be provided by the sponsor or method developer if requested by the Study Director. All contacts made during the establishment of the method will be documented in writing by the performing laboratory and presented in the final report.

Control soil samples will be fortified with known amounts of Lambda-cyhalothrin (CGA337745) and analyzed per the procedures outlined in the method (Reference 1).

One validation set should include:

- 1x Reagent Blank (matrix free sample submitted to procedures outlined in the method)
- 2x Control Samples (untreated control soil)
- 5x LOQ Recovery Samples (5 replicates at the target LOQ)
- 5x 10X LOQ Recovery Samples (5 replicates at 10X the target LOQ)

PASC should verify that the matrix control materials are free of interferences at the appropriate retention time or detector setting by examining the control samples under the instrumental conditions specified in the method. A response greater than 30% of each proposed LOQ constitutes a significant interference. If this is observed, the Study Monitor will be contacted for direction on how to proceed.

METHOD PERFORMANCE

A successful ILV will require performance data on at least one complete set of samples meeting the necessary criteria described in guidelines OECD ENV/JM/MONO (2007) 17, EPA Guideline OCSSP 850.6100 (2012), EC Guidance Documents SANCO/3029/99 Rev.4 (2000), and SANCO/825/00 Rev.8.1 (2010) of that matrix type. Generally, this requires mean recoveries to be within 70 -110% for that matrix type. The relative standard deviation of replicate measurements of recoveries should be $\leq 20\%$ at or above the method LOQ. Upon successful completion, the Study Director or delegate will proceed to write the final report. If the mean results fall outside the 70% to 110% range, the Study Director must consult the Study Sponsor to determine if further trials are required. A maximum of three (3) validation trials for that matrix type may be analyzed to show the subject method is valid.

Communication between the Study Director and the Study Sponsor should occur after each validation set is analyzed, and must be fully documented in the study records.

PROPOSED STATISTICAL METHODS

The statistical method for a regression analysis of a standard curve and quantification of residues are described in the method. The accuracy will be determined in terms of a mean and standard deviation for the recovery results from this study. Precision will be demonstrated by calculating the mean, range, standard deviation, and relative standard deviation of the sample recoveries.

MODIFICATIONS

During the 1st method trial, the method procedure should be followed as written. However, if the performance data on the first attempt is unsuccessful, the independent laboratory may contact the Study Sponsor to clarify the directions given in the method. Any correspondence must be documented in writing in the final report. If the second attempt is unsuccessful, the performing laboratory may contact the study sponsor for further clarification. Failure of the third attempt will terminate the study and a report will be issued to the study sponsor, Syngenta, explaining why the method failed.

ROUTE OF ADMINISTRATION

The test and reference material will be prepared as outlined in the analytical method.

JUSTIFICATION OF THE TEST SYSTEM

The control samples will be analyzed with the method for evaluation of substrate-related interferences, and the fortified samples will be analyzed using the method for evaluation of method performance via procedural recoveries.