1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM043.05B is suitable for the determination of lambda-cyhalothrin (Figure 1) in soil. The limit of quantitation (LOQ) of the method has been established at 0.001 mg/kg (1 ppb).

This method satisfies US EPA guidelines EPA OCSPP 850.6100, OECD ENV/JM/ MONO, SANCO/3029/99 and SANCO/825/00.

1.2 Method Summary

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

The limit of quantification of the method is 0.001 mg/kg (1 ppb).

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 lambda-cyhalothrin by one of the following methods:

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

Alternatively, the appropriate volume of hexane 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 hexane 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 lambda-cyhalothrin should be prepared by serial dilution in n-hexane from the stock solution. It is recommended that the following solutions are prepared: 10 µg/mL, 1.0 µg/mL and 0.10 µg/mL for fortification purposes.

2.3.3 Preparation of Calibration Standards for GC-MSD

Calibration standards are prepared in toluene. An aliquot from the stock solution or fortification solution can be evaporated and reconstituted using toluene for serial dilution in preparation of calibration standards. Alternatively, a stock solution can be prepared as discussed in section 2.3.1 using toluene as the dilution solvent. Using the instrumentation found in Section 4.0, the following concentration range of standards were prepared and used for calibration: 0.0002 – 0.0005 – 0.002 – 0.005 – 0.01 – 0.025 – 0.05 – 0.1 – 0.25 – 0.5 µg/mL

A calibration curve should be generated to quantify lambda-cyhalothrin residues. Standards over an appropriate concentration range should be prepared with a minimum of five levels.

Significant matrix effect was observed in some soil types, standards mentioned above were diluted 10-fold in control matrix extract. The following matrix-match calibration solutions were prepared: 0.00002 – 0.00005 – 0.0002 – 0.0005 – 0.001 – 0.0025 – 0.005 – 0.01 – 0.025 – 0.05 µg/mL where instrument sensitivity was achievable.

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 lambda-cyhalothrin 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

	n-Hexane	Acetonitrile	dichloromethane	Toluene
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-D	SHC-C, S
OES Short Term (mg/m^3)	3600	105	870	560
OES Long Term (mg/m^3)	1800	70	350	188

N/A not known

Syngenta Hazard Classification for lambda-cyhalothrin is SHC-D,S.. 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 as shown in Appendix 4. 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 lambda-cyhalothrin 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 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 lambda-cyhalothrin in n-hexane. 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 amount of soil (10 g) into a round bottom flask, add acetonitrile (40 mL) and extract by refluxing for 30 minutes using a reflux condenser and a heating block
b) Allow to cool to room temperature prior to transfer.
c) Transfer 2.5 mL into a polypropylene centrifuge tube and centrifuge for 5 minutes (3000 rpm).
d) Transfer 1.25 mL of the supernatant (equivalent to 0.25 g) into a screw-cap, polypropylene plastic centrifuge tube and add ultra-pure water (4 mL).
e) Add n-hexane (4 mL) and shake for 30 minutes.
f) Allow contents to settle then transfer the upper n-hexane layer to a clean polypropylene tube using a clean disposable plastic pipette.
g) Add an additional 4 mL portion of n-hexane to the remaining aqueous fraction and repeat the liquid-liquid partition step. Combine the upper n-hexane layer with the fraction collected above.
h) Evaporate the combined n-hexane fractions to a volume of approximately 1 mL under a stream of air of nitrogen, in a sample concentrator with the heating block set at 40°C
i) Place the required number of florisil cartridges onto a sample processing manifold.

j) Condition each cartridge with methanol (5 mL) through gravity, discard the eluates.

k) Condition each cartridge with 5 mL dichloromethane/hexane (5/95 v/v) through gravity, discard the eluates.

l) Add the extracts on the top of each cartridge and let percolate through each cartridge under gravity, discard the eluates.

m) Place test tubes (15 mL size) under each port in the manifold, as appropriate and eluate lambda-cyhalothrin residues with dichloromethane/hexane (40/60, v/v) (1x5 mL).

n) Evaporate the eluates to dryness under a stream of air of nitrogen with the heating block set at 40°C

o) Add toluene (1 mL) and ultrasonicate the sample for approximately 5 minutes in a bath of cold water and vortex.

p) Transfer the sample into a suitable autosampler vial and analyze by GC-MS using negative ion chemical ionization.

Note: 3.3 (d) volumes can be increased to 2.5 mL – 5 mL if instrument sensitivity is not sufficient. It is recommended to maintain the same ratio of ACN:UPW and partition ratio with hexane.

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 12 samples. One skilled analyst can complete the analysis of 12 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

For low level residue analysis it is recommended to perform sample container rinses using the elution solvent where applicable to increase procedural recoveries. It is also recommended to use disposable labware when possible to avoid cross-contamination.

The SPE procedure has been developed using cartridges from the stated manufacturer. Similar 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.

It should also be noted that if a larger than 1g, 6mL Florisil cartridge is used the wash aliquots should be increased from 5 mL to 8 mL, to insure analyte elution.

At high pH epimerization of lambda-cyhalothrin was observed. For analysis using the conditions in Section 4, a pH of 6 should be maintained.

4.0 FINAL DETERMINATION

The method has been developed for use on a Hewlett Packard 6890. The system is controlled and data is processed by Chemstation™ Software. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though 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 use.

4.1 Instrument Description

GC/MS

GC System	:	Hewlett Packard 6890
Detector	:	Hewlett Packard 5973

4.2 Chromatography Conditions

Column	:	HP-5MS (30.0m x 0.25 mm i.d)
Injection Port	:	Split
Carrier Gas	:	Helium at 1.0 mL/min
Injection Mode	:	Pulsed (pressure 30 psi)
Purge Time	:	2 minutes
Injection Volume	:	4 µL
Injector Temperature	:	275°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	-	150	1
2	20	300	1.5

Under these conditions the retention time for lambda-cyhalothrin is approximately: 9.1 min.

4.3 Mass Spectrometer Conditions (NICI)

Ionization Mode	:	Chemical (SIM)
Polarity	:	Negative
Calibration	:	AutoTune
Analyte	:	Lambda-cyhalothrin
Target Ion	:	241 *m/z*
Qualifier 1	:	205 *m/z*
Qualifier 2	:	243 *m/z*
Ion Ratio	:	100:33:13

4.4 Confirmatory Procedures for lambda-cyhalothrin

Final determination by GC/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

Residues of lambda-cyhalothrin may be calculated in mg/kg for each sample as follows:

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 20% LOQ to 40 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five levels).

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 mg/kg, as follows:

$$Residue\ (mg/kg) = \frac{Analyte\ found\ (\mu g/mL)}{Sample\ conc.\ (g/mL)}$$

Where on-column *Analyte Found* (µg/mg) is calculated from the standard calibration curve and sample concentration is the final sample concentration in g/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

Lambda-cyhalothrin residues may be calculated in mg/kg (ppm) 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 lambda-cyhalothrin at an appropriate concentration into the GC/MSD operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for lambda-cyhalothrin.

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

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

d) Calculate the lambda-cyhalothrin residues in the sample, expressed as mg/kg (ppm) using a mean standard response from each of the injections bracketing the sample as follows:

$$Residue\ (mg/kg) = \frac{Analyte\ found\ (\mu g/mL)}{Sample\ conc.\ (g/mL)}$$

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 concentration (g/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.

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

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. Provided the recovery values are acceptable they may be used to correct any residues found in the sample. 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 110% and with a relative standard deviation of $\leq 20\%$.

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.

FIGURE 1 Chemical Structure

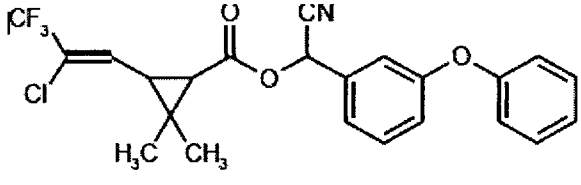
Common Name : Lambda-cyhalothrin

Code Name : PP321

CA Index Name : 1:1 mixture of (R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-(Z)-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl (1R)- cis-3-(Z)-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

Molecular Formula : $C_{23}H_{19}ClF_3NO_3$

Molecular Weight : 449.9



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
SPE	Bond Elut Florisil SPE cartridges (1 g, 6 mL) (#12256014)	www.agilent.com
Ultrasonic bath	Fisher Sci FS60H	www.fishersci.com
GC Column	HP5-MS, 30m x0.25 m, x0.25 µm	www.agilent.com

APPENDIX 2 Reagents/Chemicals

Recommended Suppliers

Reagent	Description	Supplier
Acetone	HPLC grade	www.thermoscientific.com
Dichloromethane	HPLC grade	www.thermoscientific.com
n-Hexane	HPLC grade	www.thermoscientific.com
Methanol	HPLC grade	www.thermoscientific.com
Toluene	HPLC grade	www.thermoscientific.com
Water	HPLC grade	www.thermoscientific.com
Lambda-cyhalothrin analytical standards	GLP certified	Syngenta Crop Protection, LLC

APPENDIX 3 Method Flow Chart for GC/MSD

Weigh crop sample (10 g) into 250 mL flask

↓

Add acetonitrile (40 mL) and extract by refluxing for 30 minutes.

↓

Allow to cool

↓

Transfer 2.5 mL into a centrifuge tube and centrifuge for 5 minutes (3000 rpm)

↓

Transfer 1.25 mL of the supernatant into a centrifuge tube and add ultra-pure water (4 mL)

↓

Add n-hexane (4 mL) and shake for 30 seconds

↓

Transfer the upper hexane layer to a clean tube and repeat the partition with further n- hexane

↓

Combine the hexane extracts and evaporate (at 40°C) under nitrogen flow to 1 mL

↓

Place the required number of florisil SPE cartridges onto a sample processing manifold

↓

Condition each cartridge with methanol (5 mL), discard the eluates

↓

Add 5 mL dichloromethane/hexane (5/95, v/v) onto the cartridge and draw through, discard the eluates

↓

Add the extracts on the top of each cartridge, discard the eluates

↓

Place test tubes (15 mL size) under each port in the manifold, as appropriate and eluate with (volume mL) dichloromethane/hexane (40/60, v/v)

↓

Evaporate (40°C) under nitrogen to dryness

↓

Reconstitute with toluene (1 mL)

↓

Sonicate for approximately 5 minutes, vial and submit for GC/MSD (CI Negative mode) analysis