1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM043.09A is suitable for the determination of lambda-cyhalothrin (Figure 1) in water. The limit of quantitation (LOQ) of the method has been established at 10 ng/L (10 ppt).

This method is an update to ZENECA method TMR0940B for the analysis of lambda-cyhalothrin only. The method includes updated GC-MSD conditions and decreased sample size from 500 mL to 100 mL. Fortification of water is performed using methanol.

1.2 Method Summary

100 mL of water is transferred to a 125 mL polypropylene bottle, hexane is then added equal to 5% of sample volume (5 mL). The caps are securely tightened and samples placed on a mechanical shaker for two hours. Samples are centrifuged for 3 minutes at 3500 rpm and the upper extract layer (hexane) is transferred to a 15 mL A 1.0 mL aliquot of final fraction is transferred to a GC autosampler vial. Final determination is performed by gas liquid chromatography with mass selective (GC-NICI) detection using negative ion chemical ionization.

The limit of quantification of the method is 10 ng/L (10 ppt).

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 hexanes 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 hexanes 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 methanol from the stock solution. It is recommended that the following solutions are prepared: 1.0 µg/mL, 0.10 µg/mL and 0.01 µg/mL for fortification purposes.

2.3.3 Preparation of Calibration Standards for GC-MSD

Calibration standards are prepared in hexanes. An aliquot from the stock solution can be serially diluted in preparation of calibration standards. Using the instrumentation found in Section 4.0, the following concentration of standards is recommended for calibration: 0.05 - 0.10 - 0.20 - 0.50 - 1.0 - 10.0 - 50.0 pg/µL

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.

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

	Hexanes	Methanol
Harmful Vapor	✓	✓
Highly Flammable	✓	✓
Harmful by Skin Absorption	✓	✓
Irritant to respiratory system and eyes	✓	✓
Causes severe burns	*	*
OES Short Term (mg/m^3)	3600	310
OES Long Term (mg/m^3)	70	260

Suitable personal protective equipment should be worn when handling chemicals and reagents. The appropriate MSDS should be consulted for each reagent and a local risk assessment should be carried out. In all cases avoid breathing vapour. 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

Due to the adsorptive properties of lambda-cyhalothrin and the low detection limit of the method, it is essential every precaution is taken to avoid any contamination prior to analysis.

a) Analytical procedures should be carried out in an area where no lambda-cyhalothrin has previously been extracted, if possible.

b) New disposable labware should be used where applicable

.

c) Reusable labware should be washed and solvent rinse prior to use.

d) Solvents should be checked prior to use to verify that it is free from contamination.

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 known volume of water, add the appropriate amount of standard solution containing lambda-cyhalothrin in methanol. At least one untreated control and two fortified control samples should be analyzed with each sample set.

3.3 Extraction

a) Transfer 100 mL of water to a 125 mL polypropylene bottle (Nalgene) with cap.

b) Add hexanes equivalent to 5% of the sample volume, 5 mL for a 100 mL sample and securely tighten cap.

c) Place sample on a mechanical shaker using a recommended setting to ensure complete agitation and proper partitioning. Shake sample for 2 hours.

d) Centrifuge sample at 3500 rpm for 30 minutes.

e) Using a disposable pipette, transfer the hexanes (5 mL upper organic layer) into a 15 mL polypropylene centrifuge tube.

f) Transfer a 1.0 mL aliquot of sample into a suitable autosampler vial and analyze by GC-NICI using negative ion chemical ionization.

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 13 samples. One skilled analyst can complete the analysis of 13 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 partitioning solvent where applicable to increase procedural recoveries. It is also recommended to use disposable labware when possible to avoid cross-contamination.

At high pH epimerization of lambda-cyhalothrin may be observed. For analysis using the conditions in Section 4, a pH of 6 should be maintained. If epimerization is observed by the presence of two peaks (diastereoisomer of lambda-cyhalothrin R157836 and lambda-cyhalothrin), quantitation should be performed using the sum of both peak areas.

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.

Note: Under high temperature GC-MS operating conditions, lambda-cyhalothrin may epimerize to form its diastereoisomer R157836 so that 2 peaks are sometimes observed in chromatograms of lambda-cyhalothrin. The ratio of these isomeric pairs may vary from analysis to analysis, but is constant throughout any particular analytical batch. If this is observed, both diastereoisomers should be measured and added together for a total lambda-cyhalothrin residue. This approach will require identification and retention time establishment of R157836.

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	:	Splitless / 4mm carbofrit gooseneck liner
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: 9.2 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	:	100:80:30

Representative chromatograms are shown in the Figures Section.

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 ng/L 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).

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 ng/L, as follows:

$$\text{Residue (ng/L)} = \frac{\text{Analyte found (ng/L)}}{\text{Sample conc. (mL/mL)}}$$

Where on-column *Analyte Found (*ng/L*)* is calculated from the standard calibration curve and sample concentration is the final sample concentration in mL/mL

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

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

5.2 Single Point Calibration Procedure

Lambda-cyhalothrin residues may be calculated in ng/L (ppt) 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:

$$\text{Residue (ng/L)} = \frac{\text{Analyte found (ng/L)}}{\text{Sample conc. (mL/mL)}}$$

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 or contamination 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 120% 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.

7.1 Matrix

GC/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

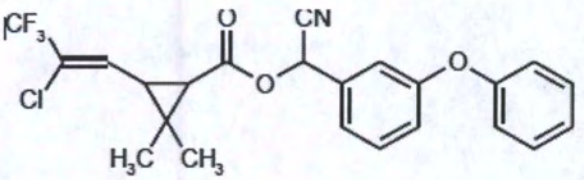
Common Name : Lambda-cyhalothrin

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
GC Column	HP5-MS, 30m x0.25 m, x0.25 µm	www.agilent.com

APPENDIX 2 Reagents/Chemicals

Recommended Suppliers

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

APPENDIX 3 Method Flow Chart for GC/MSD

Measure water sample (100 mL) into 125 mL poly bottle

↓

Add hexanes (5 mL or 5% of total volume) and extract by shaking for 2 hours

↓

Centrifuge sample at 3500 rpm for 3 minutes

↓

Transfer hexanes layer (top portion) into a 15 mL poly tube

↓

Vial and submit for GC/MSD (CI Negative mode) analysis