



**GRM043.01A**

**Analytical Method for the Determination of Total  
Cyhalothrin Residues in Crop Commodities by LC-MS/MS**

**Method**

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**EFFECTIVE DATE:** December 8, 2008

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All.

Wlodek called me with a question. The details are below. This may be noted by other labs and other protocols or in QA. Please keep this explanation on file. It has been provided to the UFL lab. Please would you coordinate informing other labs who are analyzing lambda-cyhalothrin.

Dirk Drost

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Hello IR4 Folks at UF

Upon further review concerning the cyhalothrin method, we have concluded that the standard that you received CGA134669 (now referred to as CGA337745) is the correct standard. It looks like the problem is a typo in method GRM043.01A that refers to cyhalothrin as CGA134699. The method will be reissued at some date probably using the CGA337745 designation, but for now it looks like you should be good to proceed knowing that the method refers to the wrong compound name. Please let me know if you have any more questions or concerns with this or other Syngenta methods.

Thanks, Tim



Tim Oakes

Operator and Consumer Safety

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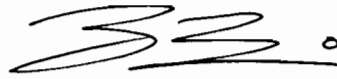
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**Version      Summary of revisions**

GRM043.01A New method - Not Applicable.

**Author:**

**Signed by:**



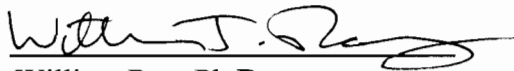
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## Abbreviations and symbols

Abbreviation	Definition
A	Acre
a.i.	active ingredient
Amt	Amount
Amu	atomic mass unit
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
Cm	Centimeter
DA[#]A	days after application, [#] = 1, 2, 3 etc., if there are multiple applications
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
Ft	foot (feet)
g	Gram
Gal	Gallon
GC	gas chromatography
GLP	Good Laboratory Practice
GRM	Global Residue Method
Ha	Hectare
HPLC	high performance liquid chromatography
i.d.	inside diameter
ID	Identification
In	Inch
IUPAC	International Union of Pure and Applied Chemistry
Kg	Kilogram
L	Liter
Lb	Pound
LC	liquid chromatography
LC-MS	tandem liquid chromatography/mass spectrometry
LC-MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	Meter

### Abbreviations and symbols (continued)

Abbreviation	Definition
µg	microgram
µL	Microliter
µm	Micrometer
MDL	method detection limit
Mg	Milligram
mL	Milliliter
Mm	Millimeter
Mmol	Millimole
Min	minute
Mol	Mole
MS	mass spectrometry
MS/MS	tandem mass spectrometry/mass spectrometry
Ms	Millisecond
mV	Millivolt
MW	molecular weight
<i>m/z</i>	mass to charge ratio
n/a	not applicable
ND or nd	not detectable (below limit of detection)
Ng	Nanogram
No.	Number
Oz	Ounce
PMRA	Pest Management Regulatory Agency, Canada
Ppb	parts per billion or micrograms per kilogram
Ppm	parts per million or microgram per gram or milligrams per kilogram
Pg	Picogram
Psi	pounds per square inch
QAU	quality assurance unit
R <sup>2</sup> (or r <sup>2</sup> )	square of correlation coefficient
RSD	relative standard deviation
Rt	retention time
s	Second
SD	standard deviation
SPE	solid phase extraction

**Abbreviations and symbols (continued)**

<b>Abbreviation</b>	<b>Definition</b>
USDA	United States Department of Agriculture
UV	Ultraviolet
Vol	Volume
Wt	Weight
V	Volt

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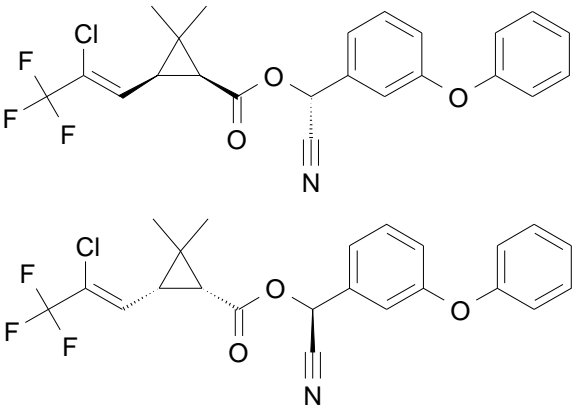
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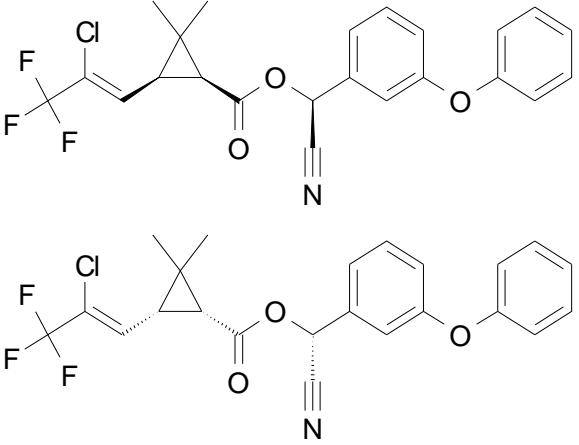
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## 1.0 INTRODUCTION

### 1.1 Scope and chemical structures

Analytical Method GRM043.01A is suitable for the determination of total cyhalothrin residues ( lambda-cyhalothrin and R157836) in crop commodities and processed products. This method is based on the extraction procedures outlined in ICI Plant Protection Division Residue Analytical Method No. 81 (Reference 1) but replaces the GC/NPD system with the HPLC-MS/MS technology for final determination of cyhalothrin residues. This method also supersedes ICI Residue Analytical Method No. 81 because (1) minimal sample cleanup effort is needed for analysis of crop matrices, resulting in considerable time saving for sample analysis, and (2) more reliable tandem mass spectrometry technology is used for accurate residue quantitation. The limit of quantitation (LOQ) of the method has been established at 0.001 ppm (mg/kg) for processed products, and at 0.01 ppm (mg/kg) for other crop commodities. This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guideline OPPTS 860.1340. The chemical structures of lambda-cyhalothrin and R157836 are summarized as follows:

<b>Compound</b>	<div style="text-align: right;">Chiral</div> 
<b>Common Name:</b>	Lambda-Cyhalothrin
<b>Code Name:</b>	R119321
<b>IUPAC Name:</b>	<i>(R)</i> -α-cyano-3-phenoxybenzyl (1 <i>S</i> )- <i>cis</i> -3-[( <i>Z</i> )-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and ( <i>S</i> )-α-cyano-3-phenoxybenzyl (1 <i>R</i> )- <i>cis</i> -3-[( <i>Z</i> )-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate
<b>CAS Name:</b>	Cyclopropanecarboxylic acid, 3-[(1 <i>Z</i> )-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethyl-, ( <i>R</i> )-cyano(3-phenoxyphenyl)methyl ester, (1 <i>S</i> ,3 <i>S</i> )-rel-
<b>CAS Number:</b>	91465-08-6
<b>Molecular Formula:</b>	$C_{23}H_{19}ClF_3NO_3$
<b>Molecular Weight:</b>	449.9

<b>Compound</b>	<div style="text-align: right;">Chiral</div> 
<b>Common Name:</b>	Epimer of Lambda-cyhalothrin
<b>Code Name:</b>	R157836
<b>IUPAC Name:</b>	<i>(S)</i> - $\alpha$ -cyano-3-phenoxybenzyl <i>(1S)</i> - <i>cis</i> -3-[( <i>Z</i> )-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and <i>(R)</i> - $\alpha$ -cyano-3-phenoxybenzyl <i>(1R)</i> - <i>cis</i> -3-[( <i>Z</i> )-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate
<b>CAS Name:</b>	Cyclopropanecarboxylic acid, 3-[(1 <i>Z</i> )-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethyl-, ( <i>S</i> )-cyano(3-phenoxyphenyl)methyl ester, (1 <i>S</i> ,3 <i>S</i> )-rel-
<b>CAS Number:</b>	91465-07-5
<b>Molecular Formula:</b>	C <sub>23</sub> H <sub>19</sub> ClF <sub>3</sub> NO <sub>3</sub>
<b>Molecular Weight:</b>	449.9

## 1.2 Method Summary

Analytical Method GRM043.01A is used for analysis of total cyhalothrin residues (lambda-cyhalothrin and R157836) in processed product and other crop commodities. For analysis of processed products such as orange juice, a ten-gram (10 g) subsample is homogenized in 200 mL of 60/40 H<sub>2</sub>O/acetonitrile (v/v) using a Polytron homogenizer. The sample extract is centrifuged or filtered with 0.45  $\mu$ m glass fiber filter and then injected onto a reverse phase HPLC-MS/MS system for analysis. For crop commodities, a ten-gram (10 g) subsample is homogenized in 200 mL acetone/hexane using a Polytron homogenizer. A 1-mL aliquot is evaporated to complete dryness and reconstituted in 10 mL of 60/40 H<sub>2</sub>O/ACN to yield a final fraction. The final solution is injected onto a reverse phase HPLC-MS/MS system for analysis of total cyhalothrin. For crop commodities such as forage and hay, an additional C<sub>18</sub> SPE cleanup procedure is needed after a 4-mL aliquot is evaporated to dryness. Since lambda-cyhalothrin and R157836 have the same retention times and instrument response factors under the HPLC-MS/MS conditions specified in this method, the residues will be analyzed as one single compound “total cyhalothrin” using CGA134699 as the external

calibration standard. CGA134699 is a ~1:1 mixture of lambda-cyhalothrin and R157836, and behave the same as lambda-cyhalothrin and R157836 under the HPLC-MS/MS conditions. The limit of quantitation (LOQ) is 0.001 ppm (mg/kg) for processed products, and 0.01 ppm (mg/kg) for crop commodities. The limit of detection (LOD) is 1 pg for CGA134699.

## **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.

Prepare 100 µg /mL primary stock solutions for CGA134699 (~1:1 lambda cyhalothrin: R157836) by one of the following methods.

The first method is to weigh 10 mg of CGA134699 (corrected for purity) into a weighing dish and quantitatively transfer (using ACN) to a “Class A” volumetric flask (100 mL). Add additional ACN to the mark on the flask.

Alternatively, the appropriate volume of ACN 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 acetonitrile required  
 W = Weight, in mg, of the solid analytical standard  
 C = Desired concentration of the final solution, ( $\mu\text{g}/\text{mL}$ )  
 1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

Sample fortification solutions should be prepared in ACN from the 100  $\mu\text{g}/\text{mL}$  CGA134699 primary stock solution. Prepare a 1  $\mu\text{g}/\text{mL}$  CGA134699 fortification standard by adding 1 mL of the primary stock solution prepared as instructed above to a 100 mL volumetric flask and diluting to the mark with ACN. Prepare a 0.1  $\mu\text{g}/\text{mL}$  CGA134699 fortification standard solution by adding 10 mL of the 1.0  $\mu\text{g}/\text{mL}$  fortification standard to a 100 mL volumetric flask and diluting to the mark with ACN. The preparation of HPLC-MS/MS calibration standards is discussed in Section 3.7. **Note: Shake these standard solutions vigorously prior to use.**

Store each solution refrigerated in the dark. Standard solutions should be allowed to equilibrate to room temperature prior to use. It is recommended that standard solutions expire 6 months after preparation.

## 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 2).

### Reagent hazards

Solvent	MeOH	Formic Acid	Acetonitrile	Hexane
Harmful Vapor	✓	✓	✓	✓
Highly Flammable	✓	X	✓	✓
Harmful by Skin Absorption	✓	✓	✓	✓
Irritant to Respiratory system & eye	✓	✓	✓	✓
Syngenta Divisional Toxicity Class	SHC-C,S	SHC-C,S	SHC-C,S	SHC-C,S
OES Short Term ( $\text{mg m}^{-3}$ )	310	N/A	105	3600
OES Long Term ( $\text{mg m}^{-3}$ )	260	9	70	70

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

At present there are insufficient data available to assign a Syngenta toxicity classification for cyhalothrin. It should be treated as a category SHC-C until further information indicates otherwise.

### **3.0 ANALYTICAL PROCEDURE**

#### **3.1 Modifications and Potential Problems**

None.

#### **3.2 Sample Preparation**

After receipt, store samples frozen at -20 °C. See details in the most current revision of Syngenta SOP 7.21 “Preparation of Crop Samples for Residue Analysis”.

#### **3.3 Extraction for Analysis of Juices**

- a) Weigh out a 10-gram representative subsample into a wide-mouth 16 oz. (500 mL) amber glass jar or a 500-mL glass flask. At least one untreated control and two control samples fortified with known amounts of CGA134699 in ACN should be analyzed with each sample set to verify method performance and allow recovery corrections to be made if desired.

**Note: No control/recovery samples will be analyzed when the method is used for tolerance enforcement purpose.**

- b) Carefully measure 200 mL of 60/40 (v/v) H<sub>2</sub>O/ACN into the 500-mL jar and homogenize the mixture for 2-3 minutes using a Polytron homogenizer at medium speed.
- c) Transfer ~50 mL of the extract into a 50-mL polypropylene centrifuge tube. Centrifuge the extract for 10 minutes at medium speed to allow the supernatant to appear.
- d) Transfer ~1 mL of aliquot of the supernatant to a HPLC injection vial for HPLC-MS/MS analysis. Further dilution with 60/40 H<sub>2</sub>O/ACN is suggested if the instrument is optimized with a higher sensitivity. See Section 4.0 for instrument installation and operating conditions for analysis of cyhalothrin

#### **3.4 Extraction for Analysis of Crop Commodities**

- a) Weigh out a 10-gram representative subsample into a wide-mouth 16 oz. (500 mL) amber glass jar or a 500-mL glass flask. At least one untreated control and two control samples fortified with known amounts of CGA134699 in ACN should be analyzed with each sample set to verify method performance and allow recovery corrections to be made if desired.

**Note: No control/recovery samples will be analyzed when the method is used for tolerance enforcement purpose.**

- b) Carefully measure 200 mL of 50/50 (v/v) hexane/acetone into the 500-mL jar and homogenize the mixture for 2-3 minutes using a Polytron homogenizer at medium speed.
- c) Transfer ~50 mL of the extract into a 50-mL polypropylene centrifuge tube. Centrifuge the extract for 10 minutes at medium speed to allow the supernatant to appear. Store extract refrigerated if Section 3.5 or 3.6 can not be performed immediately.

### 3.5 Sample Work-up for Fruits, Vegetables and Grains

- a) Transfer 1.0 mL of the extract (Section 3.4c) into a second 50-mL glass concentration tube. Evaporate the sample to **complete dryness** using a rotary evaporator in a water bath at ambient temperature. **Note: Continue evaporation for additional 10-15 minutes after the liquid solution visibly disappears to ensure the complete dryness. If necessary, add MeOH to the sample residues and evaporate again for complete dryness.**
- b) Add 4 mL of ACN to the 50-mL concentration tube and sonicate for at least 5 minutes followed by 6 mL of water. For example, a final volume of 10 mL is desired for a control or recovery samples at 0.01 ppm (mg/kg) level. Further dilution with 60/40 H<sub>2</sub>O/ACN is suggested if the instrument is optimized with a higher sensitivity.
- c) Transfer ~1 mL aliquot of the sample final fraction to an injection vial for HPLC-MS/MS analysis. Sonicate the sample for 5 minutes and shake the sample vigorously before the transfer. See Section 4.0 for instrument installation and operating conditions for analysis of cyhalothrin.

### 3.6 Sample Work-up for Forage and Hay

- a) Fit an IST C<sub>18</sub> solid phase extraction (SPE) cartridge (1-g/6 mL, IST) to a SPE vacuum manifold equipped with a nylon stopcock. Pass 5 mL of ACN through the cartridge under slight vacuum and discard the eluate. Maintain the flow rate at ~2 mL/min and discard the eluate. Close the stopcock when the solvent level approaches the cartridge bed frit.
- b) Pass 5 mL of 0.1% formic acid through the cartridge under the slight vacuum. Maintain the flow rate at ~2 mL/min and discard the eluate. Close the stopcock each time when the solvent level approaches the cartridge bed frit.
- c) Transfer 4.0 mL of the extract (Section 3.4c) into a second 50-mL glass concentration tube. Evaporate the sample to **complete dryness** using a rotary evaporator in a water

bath at ambient temperature. **Note: Continue evaporation for additional 10-15 minutes after the liquid solution visibly disappears to ensure the complete dryness. If necessary, add MeOH to the sample residues and evaporate again for complete dryness.**

- d) Add 2 mL of ACN to the 50-mL concentration tube and sonicate the sample for 5 minutes to reconstitute the residues. Swirl and sonicate all sides of the centrifuge tube to ensure the residues are dissolved in the solution.
- e) Add 3 mL of formic acid (0.1%) to the sample and mix well. Load the sample onto the preconditioned SPE cartridge using a disposable Pasteur pipette and pass through the SPE cartridge under slight vacuum. Maintain the flow rate at ~2 mL/min and discard the load. Close the stopcock when the sample level approaches the cartridge bed frit.
- f) Add 4.5 mL of 80/20 MeOH/formic acid (0.1%) to the 50-mL empty concentration tube and sonicate for 2-3 minutes. Pass the rinse through the SPE cartridge under slight vacuum. Maintain the flow rate at ~2 mL/min and discard the eluate. Close the stopcock when the sample level approaches the cartridge bed frit.
- g) Sonicate and rinse the empty concentration tube again with 5 mL of water. Pass the rinse through the SPE cartridge under slight vacuum. Maintain the flow rate at ~2 mL/min and discard the eluate. Close the stopcock when the sample level approaches the cartridge bed frit.
- h) Release the vacuum of the manifold chamber and place a 50-mL polypropylene centrifuge tube under the column outlet to receive the eluate from next step.
- i) Sonicate and rinse the empty concentration tube again with 8 mL of 90/10 ACN/H<sub>2</sub>O and pass the rinse through the SPE cartridge under slight vacuum. Maintain the flow rate at ~2 mL/min and collect the eluate into the 50-mL centrifuge tube. Let the cartridge go dry at this point.
- j) Add 9 mL H<sub>2</sub>O to the tube. Dilute the sample to the 40-mL mark with 60/40 H<sub>2</sub>O/ACN to yield final fraction. For example, a final volume of 40 mL is desired for a control or recovery samples at 0.01 ppm (mg/kg) level. Further dilution is suggested if the instrument is optimized with a higher sensitivity.
- k) Sonicate the sample for ~5 minutes. Transfer an aliquot from the final fraction into an injection vial for the LC-MS/MS analysis. See Section 4.0 for instrument installation and operating conditions for analysis of cyhalothrin.

### 3.7 Time Required for Analysis

The methodology is normally performed with a batch of 5-6 samples. One skilled person can complete the analysis of 2-3 batches of samples (12-18) in 8 working hours.

### 3.8 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples may be stored in a refrigerator or at room temperature in sealed containers when the analysis cannot be completed in a single day.

### 3.9 Preparation of Calibration Standards for HPLC-MS/MS

See preparation details for CGA134699 stock solutions and fortification standards in Section 2.3. At least 5 levels of calibration standards should be prepared to develop a calibration curve for calculation of sample residues. Standards for external calibration should be prepared in 60/40 (v/v) H<sub>2</sub>O/ACN. The following is a recommended preparation procedure:

Prepare a 1 ng/mL CGA134699 calibration standard solution by mixing 1 mL of the 0.1 µg/mL CGA134699 fortification standard with 99 mL 60/40 (v/v) H<sub>2</sub>O/ACN. Prepare calibration standards containing 0.5 ng/mL, 0.2 ng/mL, 0.1 ng/mL, 0.05 ng/mL and 0.02 ng/mL by series dilution of the 1 ng/mL standard with 60/40 (v/v) H<sub>2</sub>O/ACN. **Note: Shake the standard solutions vigorously prior to use.**

Standard concentration (ng/mL)	Standard Used (ng/mL)	Volume of Standard (mL)	Final Volume (mL)
1	100	1	100
0.5	1	25	50
0.2	1	10	50
0.1	1	5	50
0.04	1	2	50
0.02	1	1	50

CGA134699 calibration standards should be stored in a refrigerator. An expiration date of six months is recommended. Typical HPLC-MS/MS chromatograms from analysis of CGA134699 standards are presented in Appendix 3.

### 4.0 FINAL DETERMINATION

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

HPLC System : PerkinElmer 200 Micro Pump I and PerkinElmer 200 Micro Pump II with 200 Autosampler  
Detector : Applied Biosystems API 4000 Triple Quadrupole with Analyst2 Software (version 1.4)

## 4.2 Chromatography conditions

HPLC Pump: Perkin-Elmer 200 LC Pump with Autosampler  
Mobile Phase A: 0.1% formic acid in H<sub>2</sub>O  
Mobile Phase B: 0.1% formic acid in MeOH  
Flow Rate: 0.30 – 0.50 mL/min  
Column: Inertsil Phenyl-3 column (50 x 2.1 mm, i.d., 3.0µm particle size), MetaChem Technologies, Inc., Part Number: 0408 050X021  
Column Oven Temp: 40°C  
Injection Vol. 50 µL  
Run Time: 18 minutes  
Detector: Applied BioSystem API 4000  
Retention Time: ~6.6 minutes

## Mobile Phase Composition

A gradient elution, using an increased percentage of organic solvent (MeOH) in the mobile phase, will be used to resolve interferences and improve separation. See the specific gradient listed below:

<u>Time (Min)</u>	<u>A% (0.1% formic acid in water)</u>	<u>B% (0.1% formic acid in MeOH)</u>	<u>Flow (mL/min)</u>	<u>Gradient curve</u>
0.0	55	45	0.30	Initial
7.0	10	90	0.30	Linear
7.5	0	100	0.30	Linear
8.5	0	100	0.50	Isocratic
13.5	0	100	0.50	Isocratic
14.5	55	45	0.30	Linear
18	55	45	0.30	Isocratic

Note: Retention times may differ depending upon the flow rate, column, and gradient used.

### 4.3 Mass spectrometer conditions

Detection: TurboSpray Ionization positive mode Multiple Reaction Monitoring (MRM) as follows:

Q1 MS	Q3 MS	Dwell Time
m/z = 450	m/z = 225	800 ms

MS/MS Conditions:

Source Temperature:	120 °C
CAD Gas:	6
Curtain Gas:	40
GS1:	10
GS2:	18
IS:	5500
Q1 Resolution:	Unit
Q3 Resolution:	Unit
Ion Energy 1:	1.0
Ion Energy 2:	1.0
CEM:	-2200
Deflector:	400
Setting Time:	100 ms
Declostering Potential:	45
Enhance Potential:	8
Collision Energy:	18
CXP:	14

Note: The MS settings above should be used as guidelines only. For optimal results, a tune should be performed by the analyst.

Data Acquisition: Raw area counts are downloaded from the Analyst 2 data collection system (Version 1.4) to the Global Residue Information Application to calculate the final results.

## 5.0 CALCULATION OF RESULTS

### 5.1 Multi Point Calibration Procedure

This method is developed using a multipoint calibrating procedure with no matrix match calibration. Residue weight concentrations may be calculated in part per million (ppm) or mg/kg for each sample as follows.

- Prepare CGA134699 standard solutions over a concentration range appropriate to the expected residues in the samples.
- Make an injection of each sample solution and measure the areas of the peaks corresponding to the analyte of interest. Calibration standard solutions should be

interspersed throughout the analysis, after a maximum of four injections of sample solutions.

- 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 chromatography peak area, x is the CGA134699 concentration in (ng/mL) in the final fraction, m is the 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. A typical calibration curve for cyhalothrin is shown in Appendix 4.

- e) Re-arrangement for x gives

$$\text{Cyhalothrin concentration (ng/mL) in final fraction} = x = \frac{y - c}{m}$$

- f) To calculate the residue weight concentration, the sample matrix concentration in final fraction must first be calculated as follows:

$$\text{Sample matrix conc (mg/mL)} = \left( \frac{\text{sample weight (g)}}{\text{extract volume}^* \text{ (mL)}} \right) \times \left( \frac{\text{aliquot volume (mL)}}{\text{final volume (mL)}} \right) \times 1000$$

\* For orange juice, the extract volume should be the sum of extraction solvent and the sample volume (10 mL)

- h) To determine the residue weight concentration (ng/mg or ppm) of cyhalothrin in the samples, use the following equation:

$$\text{Cyhalothrin residues (ppm or mg/kg)} = \frac{\text{cyhalothrin conc (ng/mL) in final fraction}}{\text{sample matrix conc (mg/mL) in final fraction}}$$

\* For orange juice, the extract volume should be the sum of extraction solvent and the sample volume (10 mL)

## 6.0 UNTREATED CONTROL AND RECOVERY EXPERIMENTS

**Note: No control or recovery samples will be needed when the method is used for tolerance enforcement purpose.**

If untreated control samples are available, untreated control samples should be analyzed for each set of samples analyzed to verify that samples are free from analyte contamination. A minimum of one control should be analyzed with each batch of samples.

At least two recovery samples (untreated samples accurately fortified with a known amount of analytes prior to extraction) should also be analyzed alongside each batch of samples. The recovery levels should be appropriate to the residue levels expected. To determine the recovery factor, first determine the concentration of the compound in the aliquot of the sample injected, and calculate the sample concentration injected using the procedures described in Section 3.0. Calculate the final residue values found in the control and fortified samples using the equations in Section 5.1 above.

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

$$R\% = \text{Residue found in sample (ppm)} - \text{Residue found in control (ppm)} / \text{procedural recovery level (ppm)}$$

Recovery efficiency is acceptable when the mean values are between 70% and 110% and with a relative standard deviation of  $\leq 20\%$ .

## **7.0 SPECIFICITY**

### **7.1 Confirmatory Transition**

The analytical procedures outlined in ICI Plant Protection Division Residue Analytical Method No. 81 and instrument conditions can be used as the confirmatory procedures for this method. See Reference 1 for details.

### **7.2 Matrix**

No ion suppression or enhancement is found for analysis of total cyhalothrin in the selected crop commodities.

### **7.3 Reagent and Solvent Interference**

Using high purity solvents and reagents, no interference should be encountered.

### **7.4 Labware Interference**

This method uses disposable labware, where possible. All reusable glassware should be detergent washed and then rinsed with in-house deionized and HYDRO™ purified water prior to use.

## **8.0 METHOD VALIDATION**

A method validation study (Study T002559-08) (Reference 3) has been carried out on the procedures described in this method for analysis of cyhalothrin in 11 matrices: orange, apple, soybean hay, soybean seeds, spinach leaves, turnip root, straw berry, wheat grain, wild rice

grain, orange juice and wheat forage. The following discussion is based on the validation data from Study T002559-08.

## **8.1 Accuracy and Precision**

For each of the 11 matrices, five untreated control samples were fortified at 0.01 ppm (mg/kg) and five at 0.1 ppm (mg/kg), respectively. For orange juice, five untreated control samples were fortified at 0.001 ppm and 0.01 ppm, respectively. The procedural recoveries ranged from 77% to 104% for a total of 110 samples with an average of  $95 \pm 5.65\%$  (RSD = 5.9%). These results demonstrate residues of cyhalothrin can be determined in crop commodities with good accuracy and reproducibility. Typical HPLC-MS/MS chromatograms from analysis of crop commodities are presented in Appendix 5.

## **8.2 Limit of Quantitation (LOQ)**

The limit of quantitation (LOQ) is defined as the lowest analyte concentration in a sample at which the methodology has been validated. Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOQ of this method has been set at 0.001 ppm (mg/kg) for analysis of processed product sample and 0.01 ppm for crop commodities.

## **8.3 Limit of Detection (LOD)**

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as four times background noise. Note that the LOD may vary between runs and from instrument to instrument. An injections of 1 pg of CGA134699 standard (0.02 ng/mL with 50  $\mu$ L injection volume), can be reliably quantitated with a signal to noise ratio of significantly greater than 4:1. These standards are below half the LOQ, enabling reliable quantitation of samples at the LOQ.

## **8.4 Detector Linearity**

For accurate quantitation of residue concentrations, analyses should be carried out within the linear range of the detector response. Linearity of the detector is assured by the development of a calibration curve with each batch injected. It has been shown that the LC-MS/MS detector responses are generally linear in the range from 1 to 50 pg injected on column for CGA134699. These are equivalent to 0.02 to 1 ng/mL of CGA134699.

## **8.5 Radiovalidation**

None.

## **9.0 LIMITATIONS**

The method has been tested on orange juice and selected crop commodities. It can reasonably be assumed that the method can be applied for other matrices not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

## **10.0 CONCLUSIONS**

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of residues of lambda cyhalothrin and R157836 in processed product and crop commodities. Only commercially available laboratory equipment and reagents are required. The analysis of 12-18 samples can be completed by one person in a day (8 working hour period). The limit of quantitation (LOQ) of the method is 0.001 ppm (mg/kg) for analysis of processed product and 0.01 ppm for crop commodities. This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guideline OPPTS 860.1340.

## 11.0 REFERENCES

1. Sapiets, A. (1984), Plant Protection Division Residue Analytical Method No. 81: “The Determination of Residues of PP321 in Crops – a gas-liquid chromatographic method using an internal standard”
2. Luxon, S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.
3. Lin, K (2008), Syngenta Final Report T002559-06, “Validation of Analytical Method GRM043.01A for the Determination of Total Cyhalothrin Residues in Crop Commodities by LC-MS/MS”

## **APPENDICES SECTION**

## **APPENDIX 1 Apparatus**

### **US suppliers**

- 1.0 Balance, top loading, capable of actually weighing 10.00 grams, Mettler Cat. #PE-600, or equivalent;
- 2.0 Wide-mouth amber glass jar; 16 oz.
- 3.0 SPE cartridge, C<sub>18</sub> (EC), Isolute®, 1g/6mL, IST, Cat#221-0100-C or equivalent;
- 4.0 Multiple port solid phase extraction vacuum manifold equipped with nylon stopcocks, Visiprep™ 24, Supelco Cat. #5-7250-U, or equivalent;
- 5.0 Multiple port N-Evap™116, Organomation Associates, Inc., or equivalent;
- 6.0 Vial for HPLC injection, Wheaton 2-mL glass, or equivalent;
- 7.0 Disposable Volumetric pipettes, 1, 2, 5, 10 –mL;
- 8.0 Disposable Pasteur pipettes with 2-mL bulb;
- 9.0 Graduated cylinders, 10, 100, 1000-mL;
- 10.0 Volumetric flask, 50, 100-mL;
- 11.0 Polypropylene centrifuge tubes, 15-mL and 50-mL, Becton Dickinson and Company, or equivalent;

### **UK suppliers**

Unknown

## **APPENDIX 2 Reagents**

### **US suppliers**

- 1.0 Water (H<sub>2</sub>O), deionized and purified in-house with a HYDRO™ purification system or equivalent;
- 2.0 Hexane, HPLC Grade, Fisher Scientific, CAT#H3025K-4, or equivalent;
- 3.0 Acetone, HPLC Grade, Fisher Scientific, CAT#A949SK-4, or equivalent;
- 4.0 Methanol (MeOH), HPLC Grade, Fisher Scientific, CAT# AH525K-4, or equivalent;
- 5.0 Formic acid, 88%, Fisher Scientific Cat. #A118-500, or equivalent
- 6.0 Acetonitrile (ACN), HPLC Grade Fisher Scientific, CAT# A998SK-4, or equivalent;

### **Preparation of reagents**

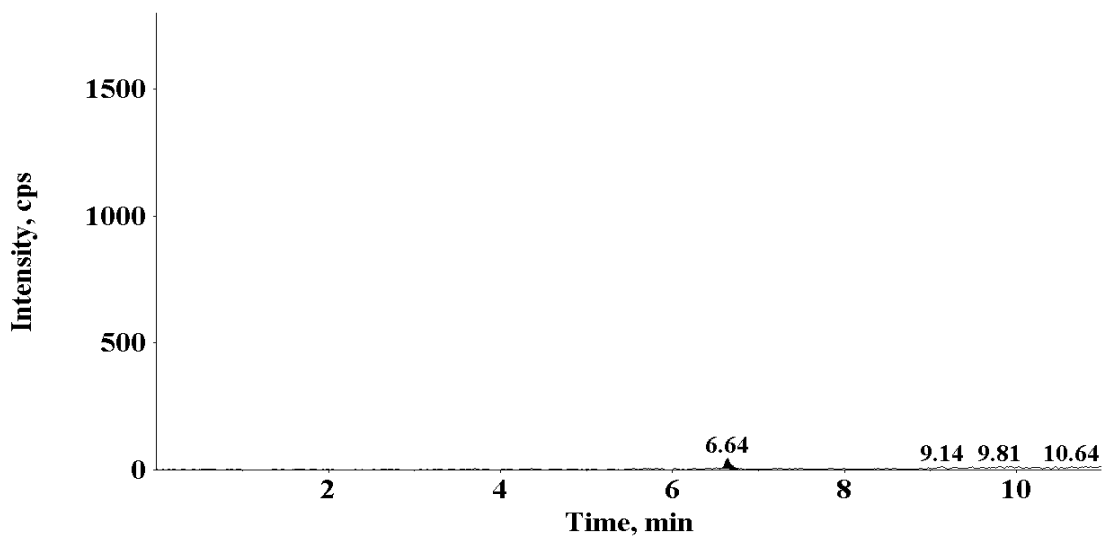
- 1.0 50/50 (v/v) acetone/hexane solution– prepared by mixing 1000 mL of acetone with 1000 mL of hexane;
- 2.0 0.1% formic acid – prepared by mixing 1.14 mL of the formic acid (88%) with 998.86 mL of water;
- 3.0 80/20 MeOH/formic acid (0.1%), prepared by mixing 400 mL of MeOH with 100 mL of 0.1% formic acid;
- 4.0 90/10 ACN/H<sub>2</sub>O, prepared by mixing 450 mL of ACN with 50 mL of water;
- 5.0 Mobile Phase A: 0.1% formic acid aqueous solution– prepared by mixing 1 mL of concentrated formic acid with 999 mL of water;
- 6.0 Mobile Phase B: 0.1% formic acid methanol solution– prepared by mixing 1 mL of concentrated formic acid with 999 mL of MeOH;

### **UK suppliers**

Unknown

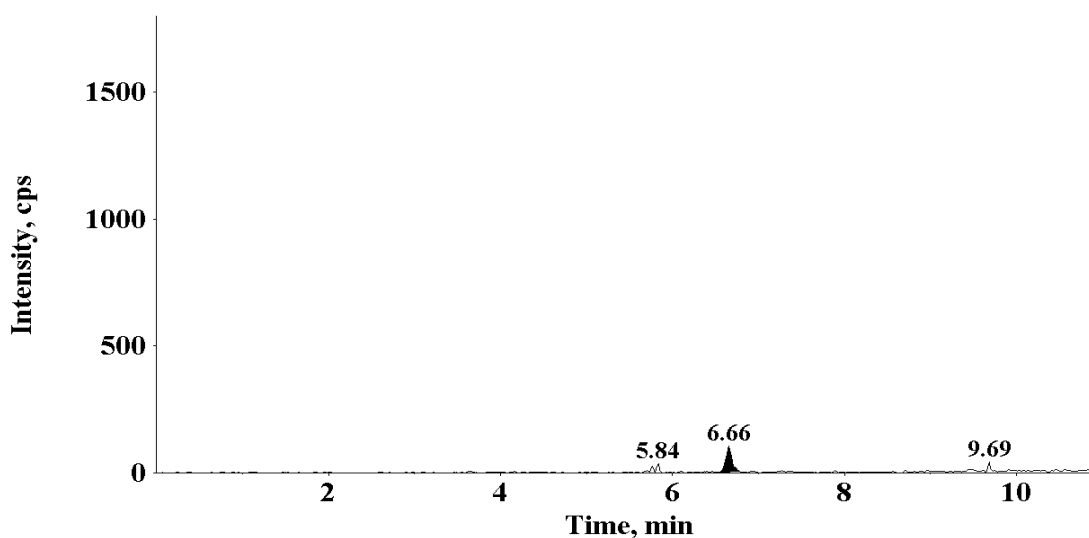
### APPENDIX 3 Typical HPLC-MS/MS Chromatograms for CGA134699 Standard

Sample003 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 3 of 12 from 10140JHSET1.wiff  
Area: 1.840e+002 counts Height: 4.04e+001 cps RT: 6.64 min



1. Standard (0.02 ng/mL), 50  $\mu$ L injection volume, 1.0 pg of CGA134699 injected, peak area = 184

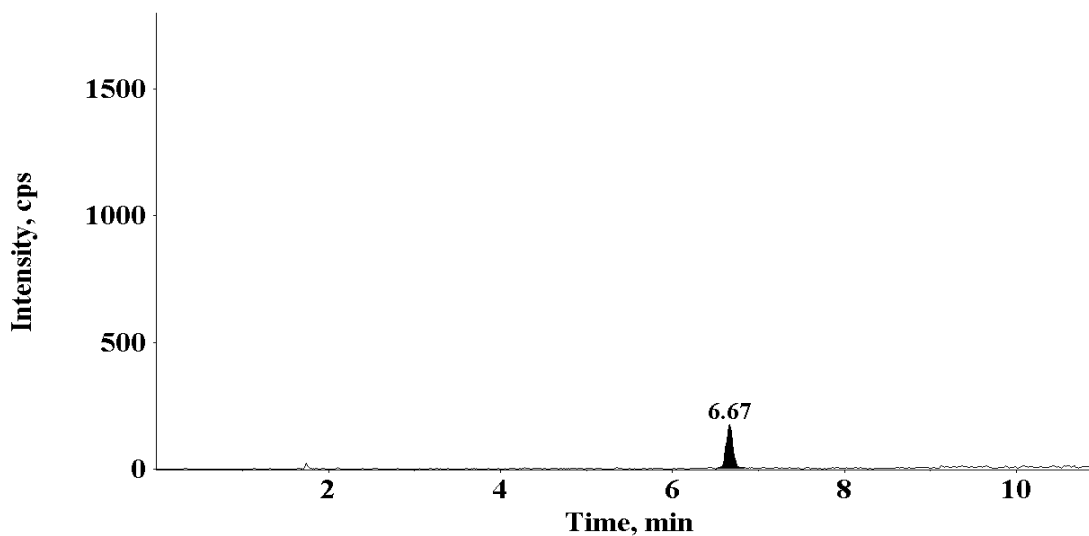
Sample010 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 10 of 12 from 10140JHSET1.wiff  
Area: 4.903e+002 counts Height: 1.01e+002 cps RT: 6.65 min



2. Standard (0.05 ng/mL), 50  $\mu$ L injection volume, 2.5 pg of CGA134699 injected, peak area = 490.3

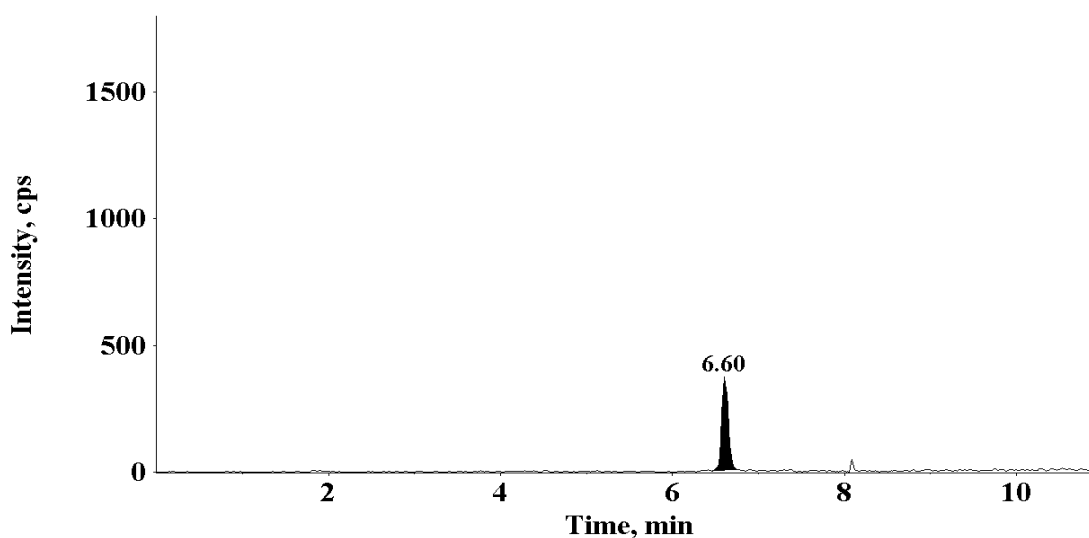
### APPENDIX 3 Typical HPLC-MS/MS Chromatograms for CGA134699 Standard (Continued)

Sample002 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 2 of 12 from 10140JHSET1.wiff  
Area: 9.509e+002 counts Height: 1.77e+002 cps RT: 6.67 min



- Standard (0.10 ng/mL), 50  $\mu$ L injection volume, 5.0 pg of CGA134699 injected, peak area = 950.9

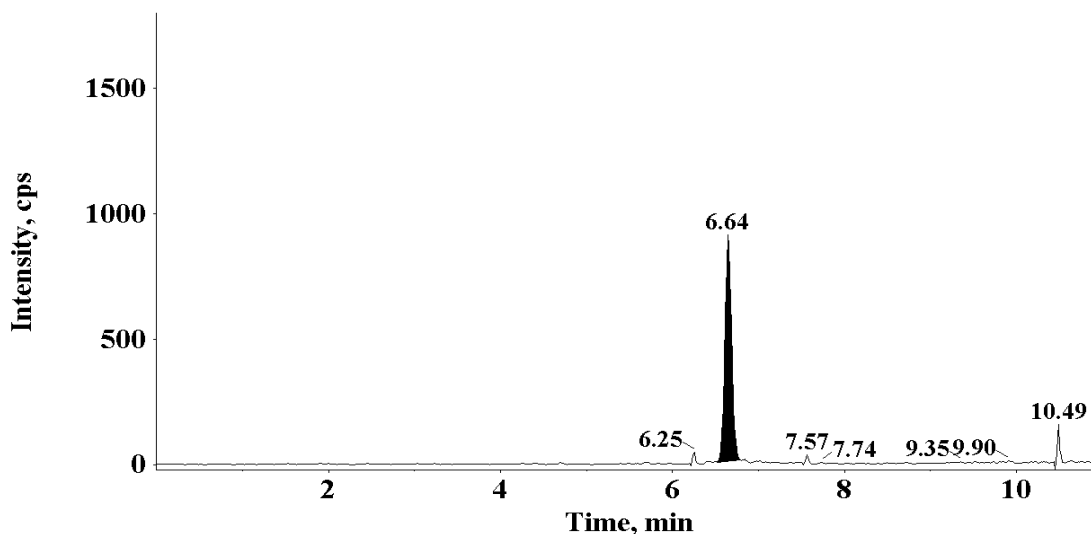
Sample011 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 11 of 12 from 10140JHSET1.wiff  
Area: 1.937e+003 counts Height: 3.70e+002 cps RT: 6.60 min



- Standard (0.20 ng/mL), 50  $\mu$ L injection volume, 10.0 pg of CGA134699 injected, peak area = 1937

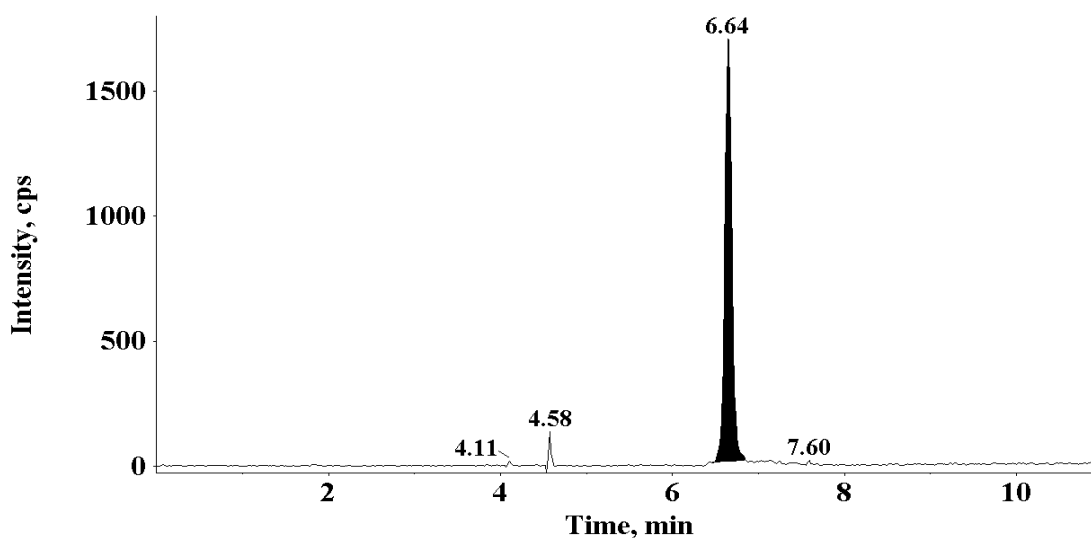
### APPENDIX 3 Typical HPLC-MS/MS Chromatograms for CGA134699 Standard (Continued)

Sample001 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 1 of 12 from 10140JHSET1.wiff  
Area: 4.882e+003 counts Height: 9.00e+002 cps RT: 6.64 min



5. Standard (0.50 ng/mL), 50  $\mu$ L injection volume, 25.0 pg of CGA134699 injected, peak area = 4882

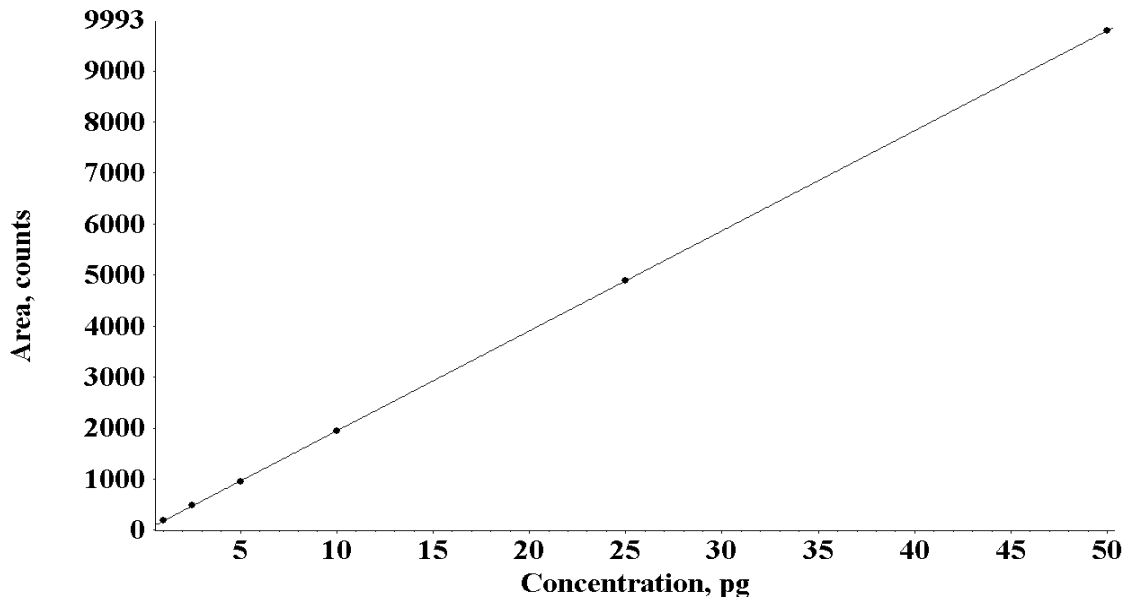
Sample012 - Cyhalothrin (Standard) 450.0/225.0 amu - sample 12 of 12 from 10140JHSET1.wiff  
Area: 9.796e+003 counts Height: 1.72e+003 cps RT: 6.65 min



6. Standard (1.0 ng/mL), 50  $\mu$ L injection volume, 50.0 pg of CGA134699 injected, peak area = 9796

## APPENDIX 4 Calibration Curve – CGA134699

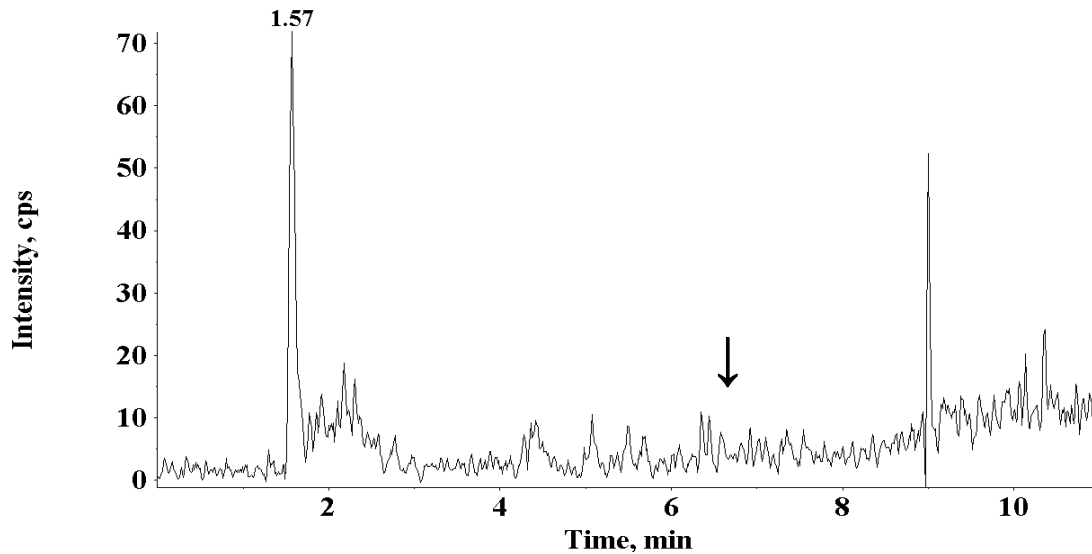
■ 1014OJH.rdb (Cyhalothrin): "Linear" Regression ("No" weighting):  $y = 196x + -17$  ( $r = 1.0000$ )



## APPENDIX 5 Typical HPLC-MS/MS Chromatograms from Analysis of Cyhalothrin Residues in Crop Matrices

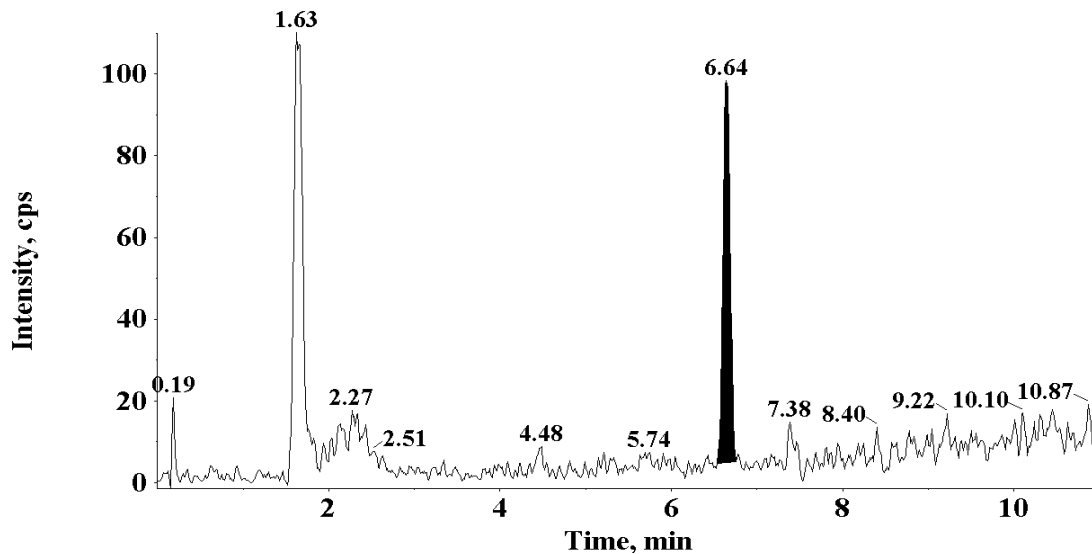
### FIGURE 1 CHROMATOGRAMS - CYHALOTHRIN IN ORANGE

Sample004 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 4 of 12 from 1009ORLSET1.wiff  
(peak not found)



1. Orange control, 0.2500 mg sample matrix injected, 0 pg of CGA134699 found, <0.01 ppm determined

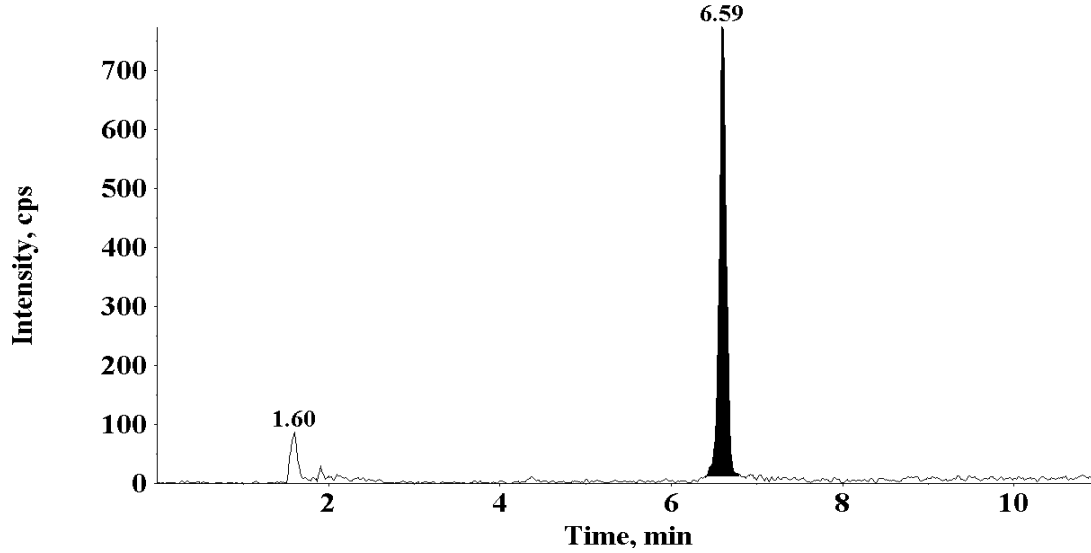
Sample009 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 9 of 12 from 1009ORLSET1.wiff  
Area: 5.598e+002 counts Height: 9.35e+001 cps RT: 6.64 min



2. Orange control + 0.01 ppm, 0.2500 mg sample matrix injected, 2.428 pg of CGA134699 found, 0.010 ppm determined, 97% recovery

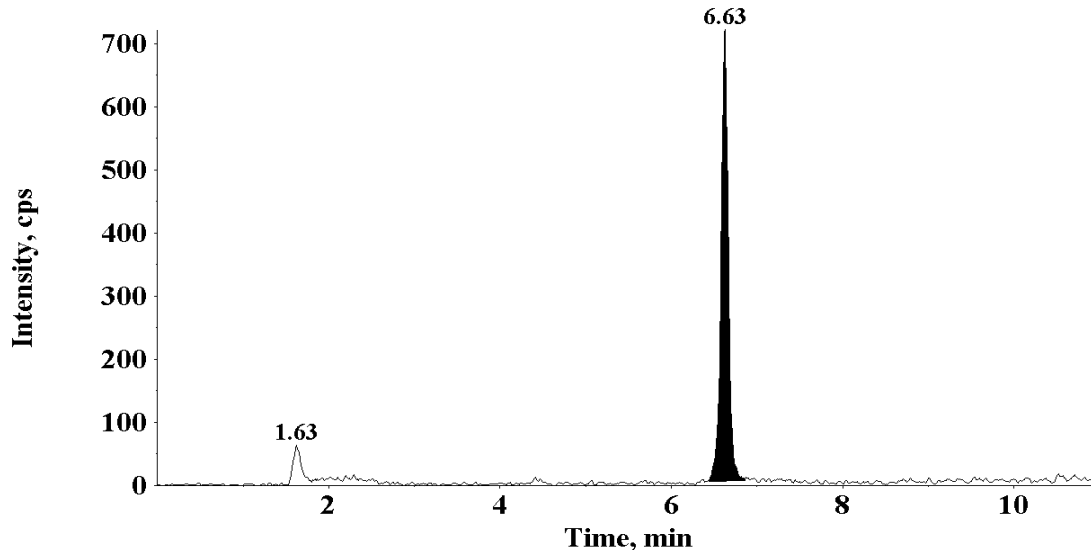
**FIGURE 1 CHROMATOGRAMS - CYHALOTHRIN IN ORANGE (Continued)**

**Sample007 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 7 of 12 from 1015ORHSET1.wiff**  
*Area: 4.349e+003 counts Height: 7.78e+002 cps RT: 6.60 min*



3. Orange control + 0.1 ppm, 0.2500 mg sample matrix injected, 25.030 pg of CGA134699 found, 0.100 ppm determined, 100% recovery

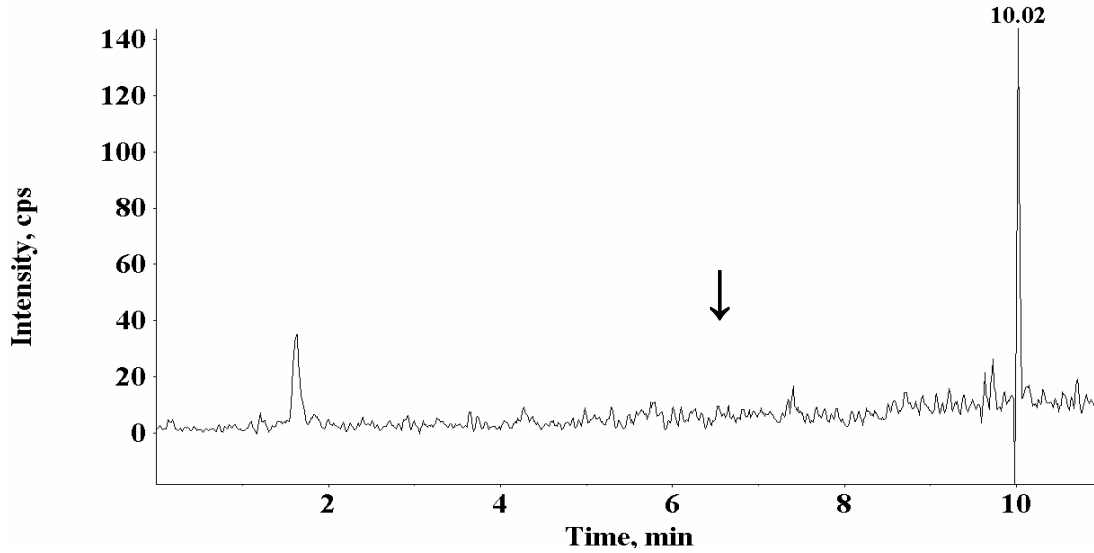
**Sample009 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 9 of 12 from 1015ORHSET1.wiff**  
*Area: 4.238e+003 counts Height: 7.15e+002 cps RT: 6.63 min*



4. Orange control + 0.1 ppm, 0.2500 mg sample matrix injected, 24.390 pg of CGA134699 found, 0.098 ppm determined, 98% recovery

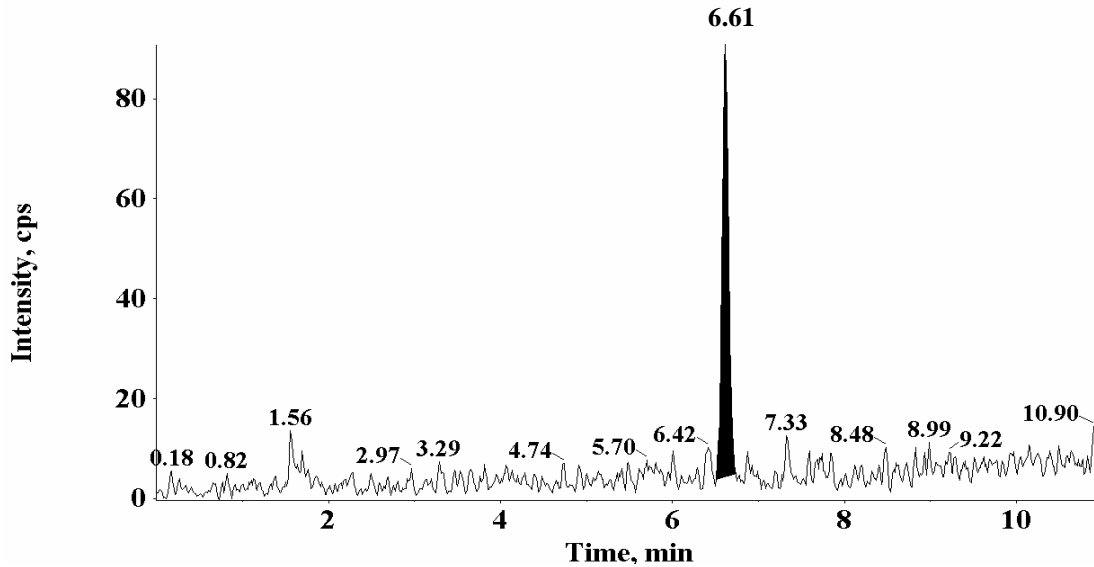
**FIGURE 2 CHROMATOGRAMS - CYHALOTHRIN IN APPLE**

**Sample004 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 4 of 12 from 0922APHSET1.wiff (peak not found)**



1. Apple control, 0.2500 mg sample matrix injected, 0 pg of CGA134699 found, <0.01 ppm determined

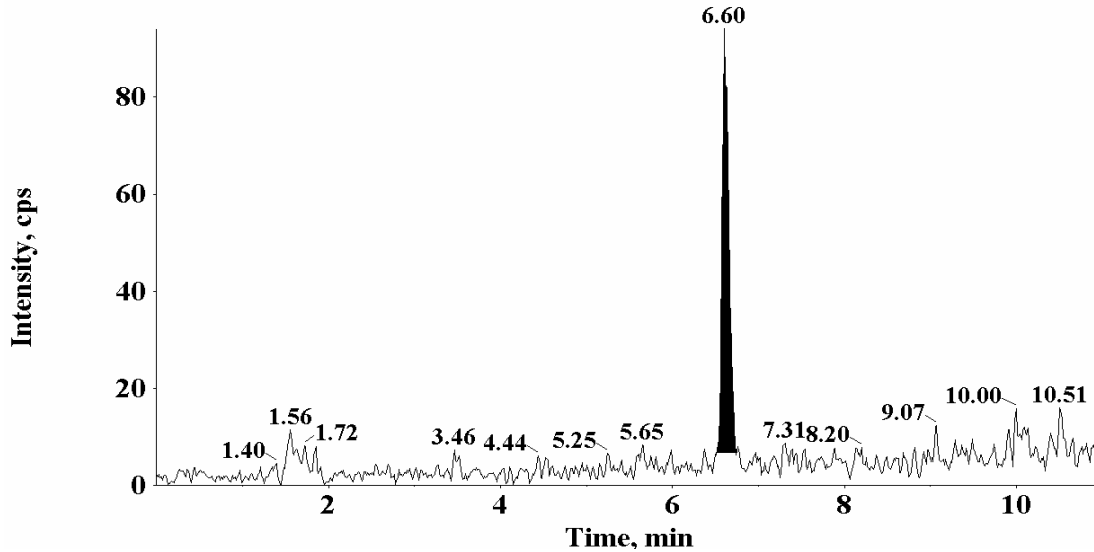
**Sample007 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 7 of 12 from 0918APSet01SET1.wiff Area: 4.530e+002 counts Height: 8.71e+001 cps RT: 6.61 min**



2. Apple control + 0.01 ppm, 0.2500 mg sample matrix injected, 2.615 pg of CGA134699 found, 0.010 ppm determined, 91% recovery

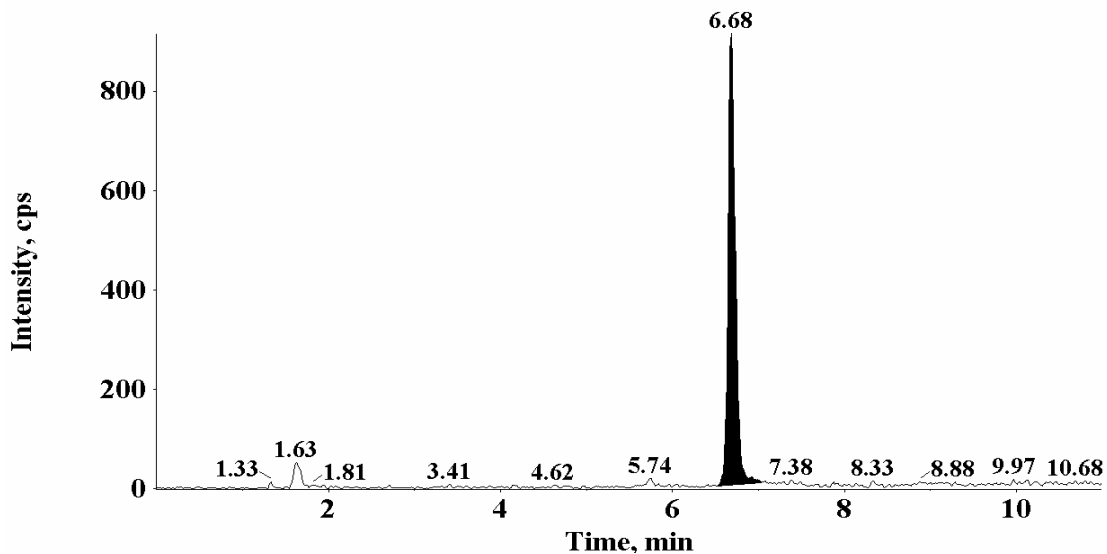
**FIGURE 2 CHROMATOGRAMS - CYHALOTHRIN IN APPLE (Continued)**

**Sample008 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 8 of 12 from 0918APSet01SET1.wiff**  
**Area: 4.621e+002 counts Height: 8.76e+001 cps RT: 6.60 min**



3. Apple control + 0.01 ppm, 0.2500 mg sample matrix injected, 2.665 pg of CGA134699 found, 0.011 ppm determined, 93% recovery

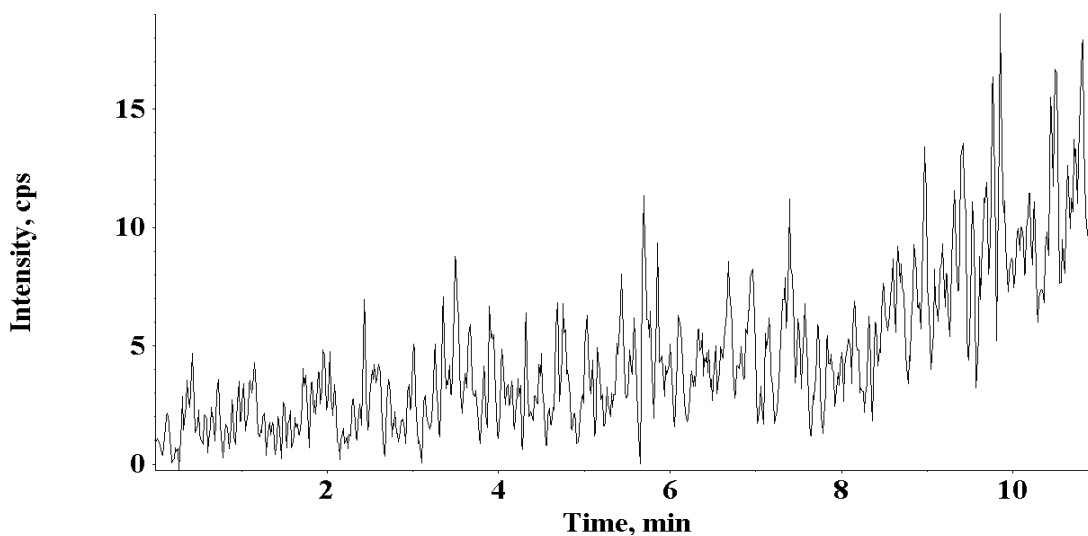
**Sample005 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 5 of 12 from 0922APHSET1.wiff**  
**Area: 5.467e+003 counts Height: 9.08e+002 cps RT: 6.68 min**



4. Apple control + 0.1 ppm, 0.2500 mg sample matrix injected, 24.80 pg of CGA134699 found, 0.099 ppm determined, 99% recovery

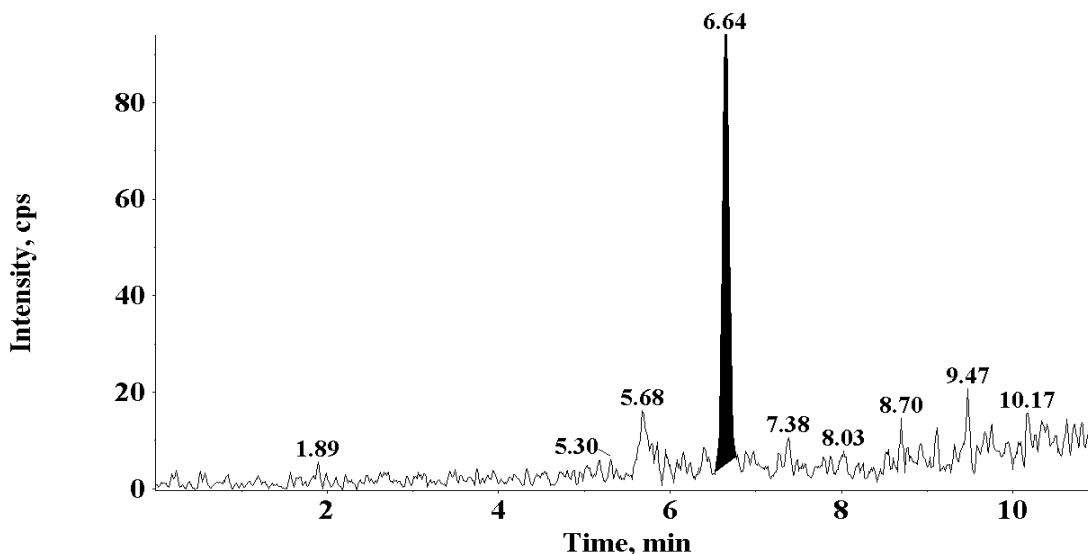
### FIGURE 3 CHROMATOGRAMS - CYHALOTHRIN IN WHEAT FORAGE

Sample004 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 4 of 12 from 1020WFHSET1.wiff  
(peak not found)



1. Wheat forage control, 0.2500 mg sample matrix injected, 0 pg of CGA134699 found, <0.01 ppm determined

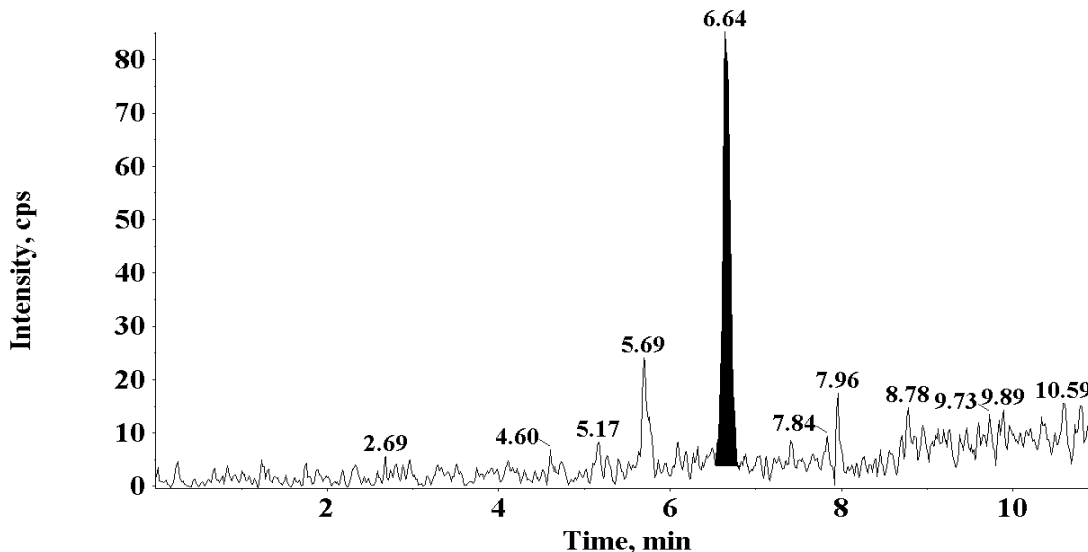
Sample005 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 5 of 12 from 1009WFLR5SET1.wiff  
Area: 5.128e+002 counts Height: 9.02e+001 cps RT: 6.65 min



2. Wheat forage control + 0.01 ppm, 0.2500 mg sample matrix injected, 2.533 pg of CGA134699 found, 0.010 ppm determined, 101% recovery

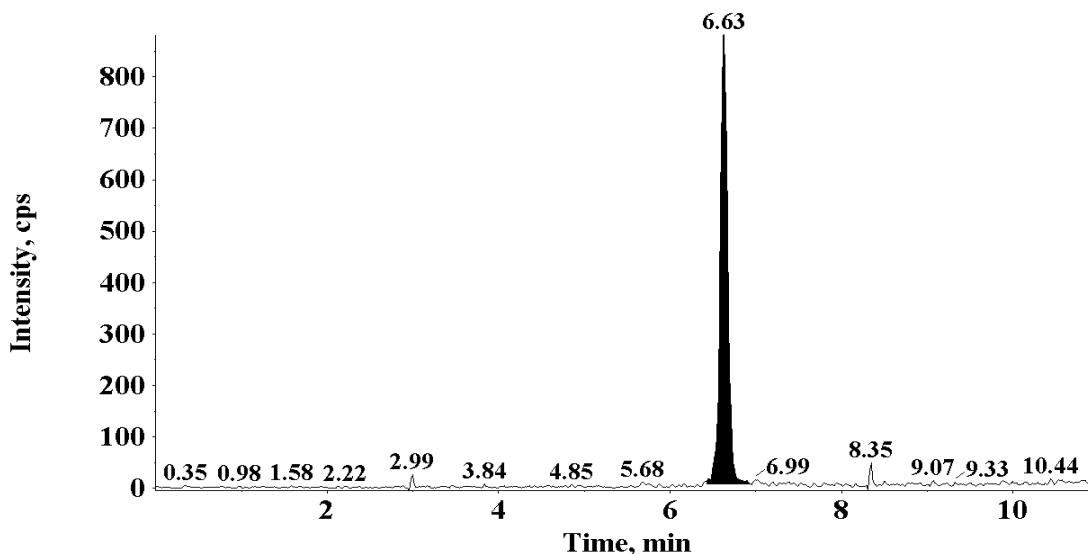
### FIGURE 3 CHROMATOGRAMS - CYHALOTHRIN IN WHEAT FORAGE (Continued)

Sample009 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 9 of 12 from 1009WFLR5SET1.wiff  
Area: 5.055e+002 counts Height: 8.16e+001 cps RT: 6.65 min



3. Wheat forage control + 0.01 ppm, 0.2500 mg sample matrix injected, 2.499 pg of CGA134699 found, 0.010 ppm determined, 100% recovery

Sample005 - Cyhalothrin (Unknown) 450.0/225.0 amu - sample 5 of 12 from 1020WFHSET1.wiff  
Area: 5.414e+003 counts Height: 8.72e+002 cps RT: 6.63 min



4. Wheat forage control + 0.1 ppm, 0.2500 mg sample matrix injected, 23.20 pg of CGA134699 found, 0.093 ppm determined, 93% recovery

## APPENDIX 6 Method Flowchart

