

# TEST REPORT

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<b>Final report</b>	
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## 1. Report to the attention of

Company : Disease Control Technologies LLC	VAT Number : N/A
Street : The Parkway N° : 419	Box :
Postal code : 29650 City : Greer, South Carolina	Country : USA
Contact Rod Flinn	E-mail : <a href="mailto:rod@diseasecontroltechnologies.com">rod@diseasecontroltechnologies.com</a>
Mobile phone : Phone : +1 (202) 465 4611	Fax :

## 2. Samples

### Nature : **Royal Guard, 120 denier**

- Formulation : long-lasting (incorporated into polyethylene) insecticidal net (LN)
- Active ingredients content (nominal concentration) : alpha-cypermethrin 5.5 g/kg  
pyriproxyfen 5.5 g/kg
- Manufacturer : Disease Control Technologies LLC
- Yarn : polyethylene 120 denier
- Colour : blue

### **Royal Guard, 150 denier**

- Formulation : long-lasting (incorporated into polyethylene) insecticidal net (LN)
- Active ingredients content (nominal concentration) : alpha-cypermethrin 5.0 g/kg  
pyriproxyfen 5.0 g/kg
- Manufacturer : Disease Control Technologies LLC
- Yarn : polyethylene 150 denier
- Colour : white

### **Peer-validation of the CIPAC method extension with IIBAT, India.**

Number : 3 net samples for each Royal Guard

Receipt date : March 29, 2017

Net No.1, Net No.2 and Net No.3

Each net sample contains 5 pieces of 25 cm x 25 cm  
taken from each side and roof of the net

Pre-treatment/storage before analysis\* : at room temperature in the original packaging  
under shelter from direct sunlight

Lab Id number : Mo 492/1 to Mo 492/6

Storage\* : 3 months after analysis

\* irrelevant if not specified

### 3. Performed analyses

Analyses	Methods or standards	Sub-contracting (name)	Accredited ISO 17025 (A)	Dates
Laboratory sampling	Pooling of the 5 pieces of each net sample, cutting into small pieces of 5-10 mm square and homogenization in order to determine alpha-cypermethrin and pyriproxyfen content.			21/04/2017 to 24/04/2017
Alpha-cypermethrin and pyriproxyfen identity and content	<p>CIPAC/4886/m (extension of CIPAC 715/TC/M/3) (HPLC-DAD) for pyriproxyfen and CIPAC/5043 (extension of CIPAC 454/LN/M/3.2, CIPAC Handbook M, page 41) (GC-FID) for alpha-cypermethrin.</p> <p>Extraction in a water bath at 85-90°C for 45 minutes with heptane in presence of dicyclohexyl phthalate as internal standard and citric acid. Determination of alpha-cypermethrin by Gas Chromatography with Flame Ionization Detection (GC-FID). Determination of pyriproxyfen by High Performance Liquid Chromatography with UV Diode Array Detection (HPLC-DAD) after solvent exchange to the mobile phase.</p> <p><u>Repeatability and intra-laboratory reproducibility</u></p> <p>Duplicate analysis (2 samples aliquots of the cut pooled net sample) on 2 different days.</p> <p><u>Linearity of the detector response</u></p> <p>5 calibration solutions</p> <p><u>Specificity</u></p> <p>1 solvent blank, 1 method blank, 1 sample blank (without internal standard) and 1 untreated sample</p>		(A)	25/04/2017 to 02/05/2017

## 4. Results

### 4.1 Analytical method for alpha-cypermethrin and pyriproxyfen in Royal Guard

#### 4.1.1 OUTLINE OF METHOD

The pyriproxyfen in LN is determined by reverse phase high performance liquid chromatography using UV detector, at detection wavelength of 254 nm, with dicyclohexyl phthalate as internal standard (extension of CIPAC 715/TC/M/3). The same sample extracted in heptane is used for the determination of alpha-cypermethrin by gas chromatography with flame ionisation detection (CIPAC 454/LN/M/3.2).

#### 4.1.2 APPARATUS

- Analytical balance (to the nearest 0.1 mg).
- Horizontal shaking and heating water bath.
- Laboratory glassware.
- High performance liquid chromatograph, equipped with an UV diode-array detector (HPLC-DAD) and a constant-temperature column oven.
- Column Phenomenex Luna C18-2, 5 µm, 100A, 250 x 4.6 mm i.d. (or equivalent material with the same selectivity).
- Gas chromatograph, equipped with a flame ionisation detector (GC-FID) and a split / splitless injection system.
- Capillary column, fused silica, 30 m x 0.25 mm i.d., 0.25 µm film thickness, coated with dimethyl polysiloxane (DB-1) (or equivalent material with the same selectivity).
- 0.2 µm nylon membrane filters.

#### 4.1.3 CONSUMABLES

- Alpha-cypermethrin, certified analytical standard of known purity (Dr. Ehrenstorfer / LGC Labor, purity 99.0%, batch 105282 and Sigma-Aldrich, purity 99.8%, batch SZBF061XV).
- Pyriproxyfen, certified analytical standard of known purity (Dr. Ehrenstorfer / LGC Labor, purity 99.0%, batch 117165).
- Dicyclohexyl phthalate, analytical reagent grade (Sigma-Aldrich, purity 99.9%, batch 09203CDV).
- Citric acid, 10 % solution : dissolve 50 g citric acid in 500 mL tetrahydrofuran.
- Heptane, analytical reagent grade.
- 2-Propanol, analytical reagent grade.
- Tetrahydrofuran, analytical reagent grade.
- Acetonitrile, HPLC grade.
- Water, HPLC grade or MilliQ® grade.

#### 4.1.4 ANALYTICAL PROCEDURE

##### 4.1.4.1 Internal standard solution

Dissolve 5 g dicyclohexyl phthalate in 200 mL of 2-propanol and use for the preparation of all calibration and sample solutions.

##### 4.1.4.2 Calibration solutions of alpha-cypermethrin

Weigh about 25, 40, 50, 75 and 100 mg (to the nearest 0.1 mg) of alpha-cypermethrin analytical standard in five 50 mL volumetric flasks. Add by pipette to each flask 5 mL internal standard solution, citric acid solution (2 mL) and fill to the mark with heptane (solutions C<sub>A</sub>, C<sub>B</sub>, C<sub>C</sub>, C<sub>D</sub> and C<sub>E</sub>) respectively. Prepare five calibration solutions comprising approximately 0.5, 0.8, 1.0, 1.5, and 2.0 times that of calibration solution.

##### 4.1.4.3 Calibration solutions of pyriproxyfen

Weigh about 25, 40, 50, 75 and 100 mg (to the nearest 0.1 mg) of pyriproxyfen analytical standard in five 50 mL volumetric flasks. Add by pipette to each flask 5 mL internal standard solution, and fill to the mark with acetonitrile (solutions C<sub>A</sub>, C<sub>B</sub>, C<sub>C</sub>, C<sub>D</sub> and C<sub>E</sub>) respectively. Prepare five calibration solutions comprising approximately 0.5, 0.8, 1.0, 1.5, and 2.0 times that of calibration solution.

#### 4.1.4.4 Sample solutions

Clean a pair of scissors with acetone before use. Cut the sample with the scissors into 5 - 10 mm squares. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 9 to 11 mg ( $w$  mg) of pyriproxyfen and 9 to 11 mg ( $w$  mg) of alpha-cypermethrin into a vial or stoppered flask (100 mL). Add by pipette internal standard solution (1.0 mL), citric acid solution (2 mL) and by measuring cylinder heptane (47 mL). Place the vial or stoppered flask in a horizontal shaking and heating water bath (85-90°C) for 45 min. Shake the vial or stoppered flask once or twice during the extraction. After extraction, cool it to room temperature (solutions  $S_A$  and  $S_B$ ). For alpha-cypermethrin, analyse an aliquot of the filtered extract directly by using GC-FID. For pyriproxyfen, transfer the extract solution by pipette (5.0 mL) into a round-bottom flask (50 mL). Evaporate the solution *in vacuo*, add by pipette acetonitrile (5.0 mL) and dissolve completely (solutions  $S_A$  and  $S_B$ ).

#### 4.1.4.5 Procedure for alpha-cypermethrin

##### (a) Operating chromatographic conditions

Chromatographic determination by GC-FID (typical)

##### APPARATUS

- Gas chromatograph : Agilent Technologies 6890 Series.
- Autosampler : Agilent Technologies 7683 Series.
- Injection system : split.
- Liner : split, single taper, glass wool, deactivated, low pressure drop.
- Detector : Flame Ionisation Detector (FID).
- Software of integration : Agilent ChemStation.

##### CHROMATOGRAPHIC PARAMETERS

- Column : capillary fused silica, 30 m x 0.25 mm i.d., 0.25  $\mu$ m film thickness, coated with 100 % methyl siloxane (DB-1) (or equivalent material with the same selectivity).
- Carrier gas : helium - 0.8 mL / minute (constant flow).
- Split ratio : 100:1.
- Make-up gas : helium - 30 mL / minute.
- Hydrogen flow to detector : 30 mL / minute.
- Air flow to detector : 300 mL / minute.
- Inlet temperature : 260°C.
- Oven temperature : 235°C for 27 minutes.
- Detector temperature : 300°C.
- Injection volume : 1  $\mu$ L.
- Runtime : 27 minutes.

Retention times with these conditions : dicyclohexyl phthalate : 10.6 minutes  
 alpha-cypermethrin (cis I) isomer : 22.0 minutes  
 alpha-cypermethrin (cis II) : 22.7 minutes.

These chromatographic conditions may be adapted in order to achieve a good chromatographic separation.

##### (b) Linearity check

Before conducting the analysis check the linearity of the detector response by injecting 1  $\mu$ L portions of five calibration solutions of alpha-cypermethrin concentrations as described hereunder. The internal standard dicyclohexyl phthalate was used while preparing the calibration solutions of alpha-cypermethrin.

##### (c) System equilibration

Inject 1  $\mu$ L portions of calibration solution  $C_C$  until the response factors obtained for two consecutive injections differ by less than 1.0%.

## (d) Calculation

Calculate the mean value of the response factors of the calibration solution bracketing two sample solutions and use this value to calculate the alpha-cypermethrin concentration of the bracketed samples.

$$fi = \frac{lr \times s \times P}{Hs}$$

$$\text{Active content} = \frac{f \times Hw}{lq \times w \times 5} \text{ g/kg}$$

Where:

<i>fi</i>	=	Individual response factor
<i>f</i>	=	Mean response factor
<i>Hs</i>	=	Peak area of active ingredient in the calibration solution
<i>Hw</i>	=	Peak area of active ingredient in the sample solution
<i>lr</i>	=	Peak area of internal standard in the calibration solution
<i>lq</i>	=	Peak area of internal standard in the sample solution
<i>s</i>	=	Mass of standard in sample solution (mg)
<i>w</i>	=	Mass of sample taken (mg)
<i>P</i>	=	Purity of standard (g/kg)

#### 4.1.4.6 Procedure for pyriproxyfen

(a) Operating chromatographic conditions

Chromatographic determination by HPLC-DAD (typical)

## APPARATUS

- Liquid chromatograph (pump) : Agilent Technologies G1312A (1200 series).
- Autosampler : Agilent Technologies G1329A (1200 series).
- Oven for column : Agilent Technologies G1316A (1100 series).
- Detector : Diode-Array Agilent G1315A (1100 series).
- Software of integration : Agilent HPLC3D ChemStation (DOS series).

## CHROMATOGRAPHIC PARAMETERS

- Column : Phenomenex Luna C18-2, 5 µm, 100A, 250 x 4.6 mm i.d. (or equivalent material with the same selectivity).
- Mobile phase: acetonitrile / water (70/30, v/v).
- Flow rate : 1 mL / minute.
- Injection volume : 10 µL.
- Column temperature : 40°C.
- Detector wavelength : 254 nm.
- Runtime : 35 minutes.

Retention times with these conditions : pyriproxyfen : 16.7 minutes  
dicyclohexyl phthalate : 28.6 minutes.

These chromatographic conditions may be adapted in order to achieve a good chromatographic separation.

## (b) Linearity check

Before conducting the analysis check the linearity of the detector response by injecting 10 µL portions of five calibration solutions of pyriproxyfen concentrations as described hereunder. The internal standard dicyclohexyl phthalate was used while preparing the calibration solutions of pyriproxyfen.

## (c) System equilibration

Inject 10 µl portions of calibration solution  $C_c$  until the response factors obtained for two consecutive injections differ by less than 1.0%.

## (d) Calculation

Calculate the mean value of the response factors of the calibration solution bracketing two sample solutions and use this value to calculate the pyriproxyfen concentration of the bracketed samples.

$$f_i = \frac{l_r \times s \times P}{H_s}$$

$$\text{Active content} = \frac{f \times H_w}{l_q \times w \times 5} \text{ g/kg}$$

Where:

- $f_i$  = Individual response factor
- $f$  = Mean response factor
- $H_s$  = Peak area of active ingredient in the calibration solution
- $H_w$  = Peak area of active ingredient in the sample solution
- $l_r$  = Peak area of internal standard in the calibration solution
- $l_q$  = Peak area of internal standard in the sample solution
- $s$  = Mass of standard in sample solution (mg)
- $w$  = Mass of sample taken (mg)
- $P$  = Purity of standard (g/kg)

## 4.2 Method assessment

### 4.2.1 Specificity and non-analyte interference

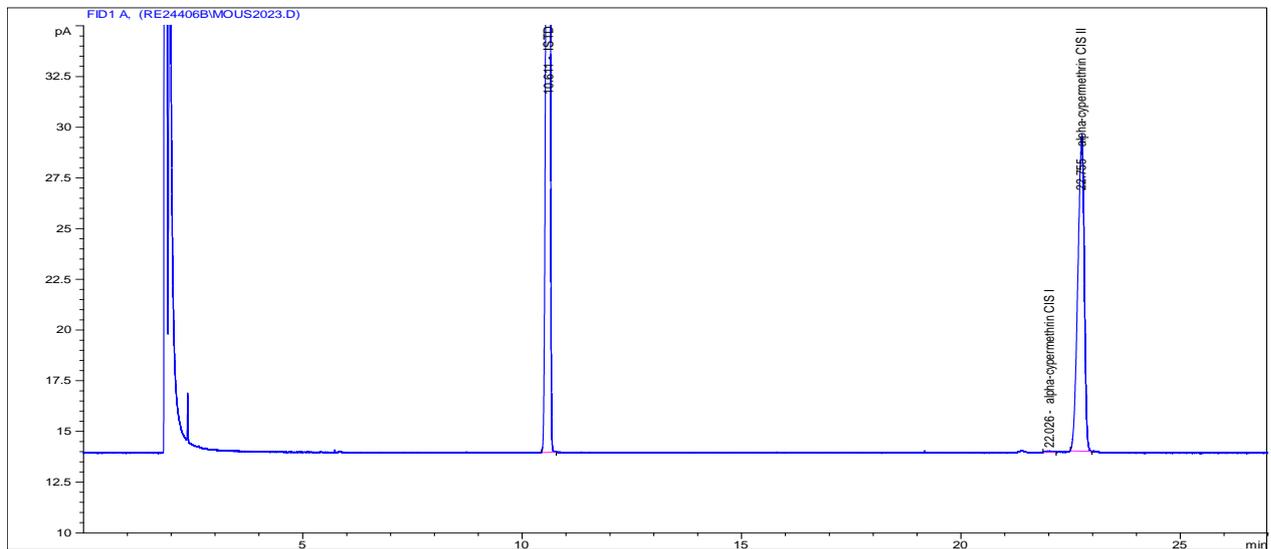
#### Alpha-cypermethrin by GC-FID

The retention time of the alpha-cypermethrin (cis I) isomer, alpha-cypermethrin (cis II) and dicyclohexyl phthlate (internal standard) peaks by GC-FID in the sample solutions does not deviate by more than 1.0% from that of the calibration solutions.

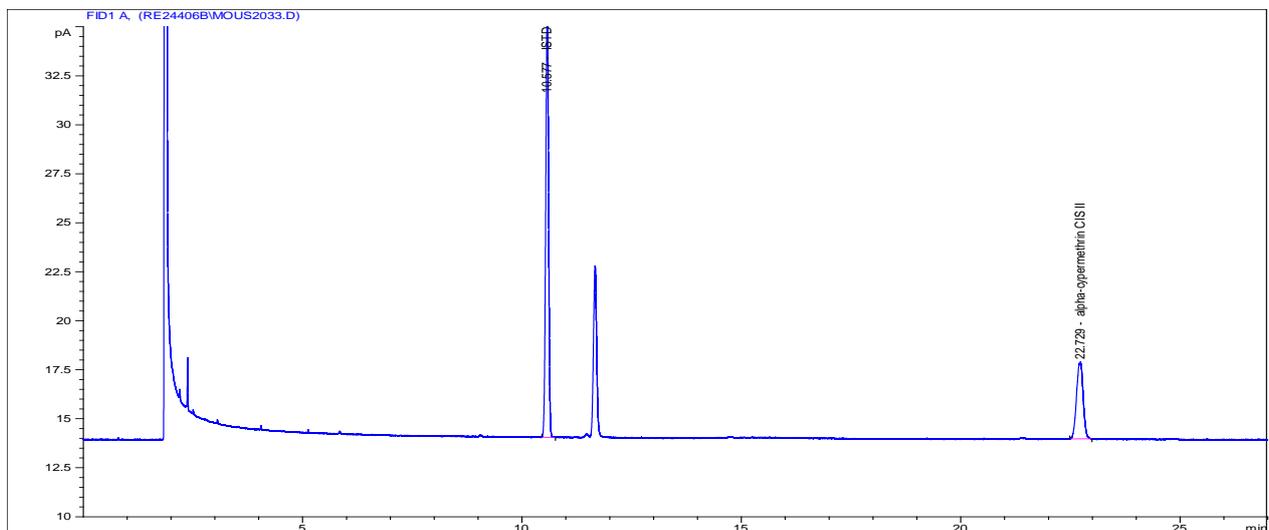
The analysis of blanks (solvent blank, method blank, sample without internal standard) and untreated blank samples in comparison with the analysis of calibration solutions and treated samples showed the absence of compound interfering with the determination of alpha-cypermethrin (cis I) isomer, alpha-cypermethrin (cis II) and dicyclohexyl phthlate.

The specificity and non-analyte interference meet therefore the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

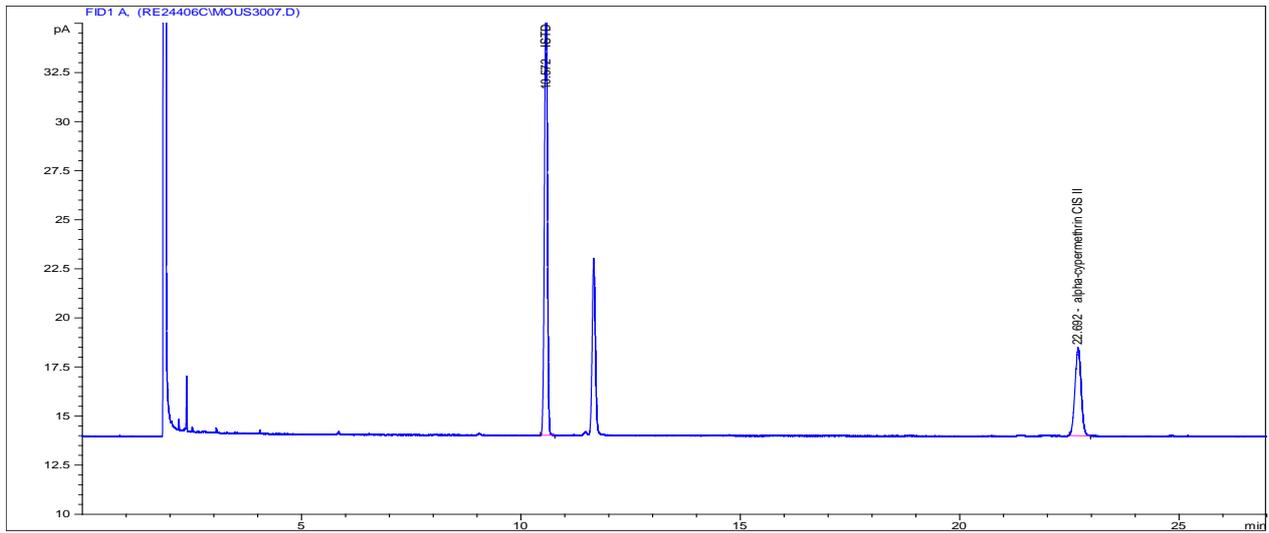
#### Representative chromatograms for alpha-cypermethrin by GC-FID



Alpha-cypermethrin calibration solution



Royal Guard, 120 denier sample solution



Royal Guard, 150 denier sample solution

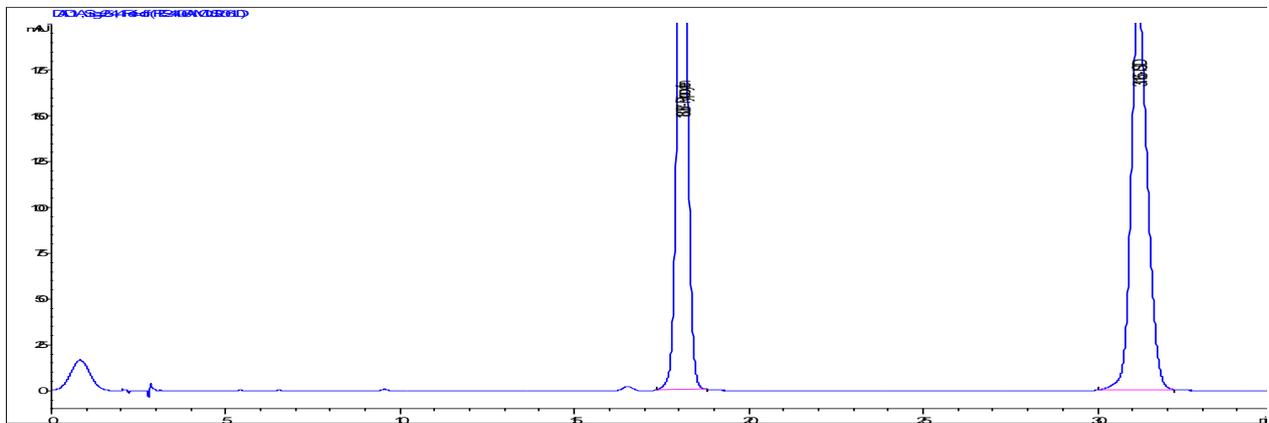
Pyriproxyfen by HPLC-DAD

The retention time of the pyriproxyfen and dicyclohexyl phthlate (internal standard) peaks by HPLC-DAD in the sample solutions does not deviate by more than 1.0% from that of the calibration solutions.

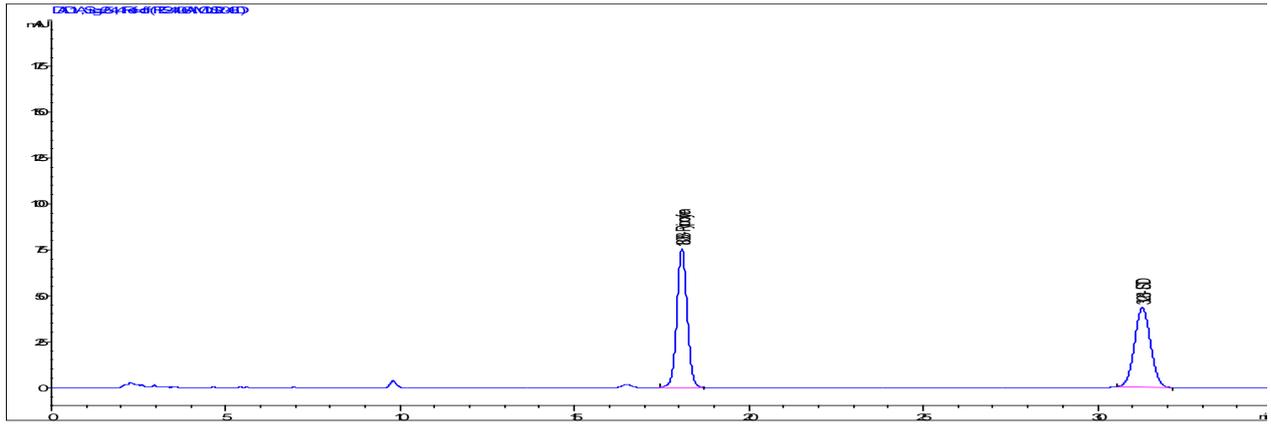
The analysis of blanks (solvent blank, method blank, sample without internal standard) and untreated blank samples in comparison with the analysis of calibration solutions and treated samples showed the absence of compound interfering with the determination of pyriproxyfen and dicyclohexyl phthlate.

The specificity and non-analyte interference meet therefore the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

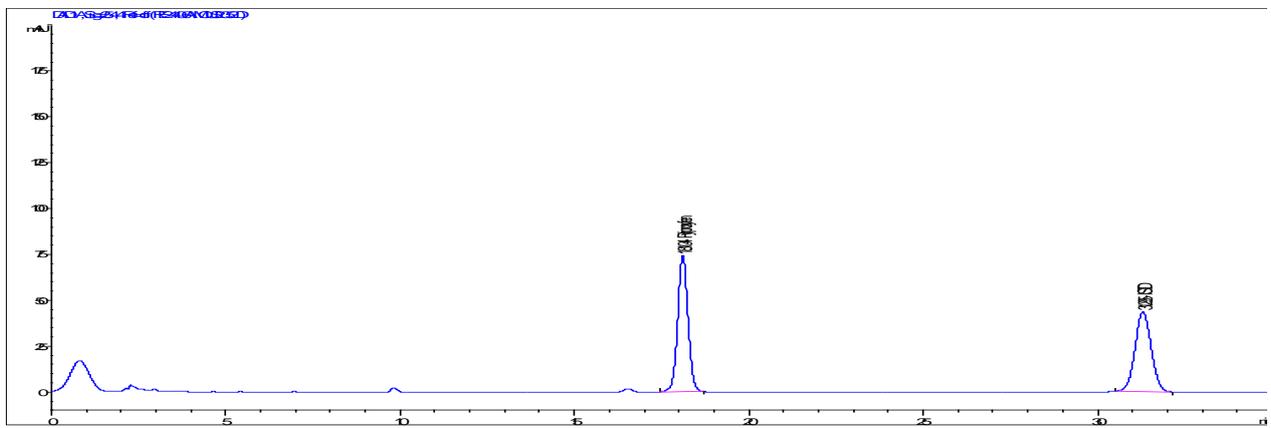
Representative chromatograms for pyriproxyfen by HPLC-DAD



Pyriproxyfen calibration solution



Royal Guard, 120 denier sample solution



Royal Guard, 150 denier sample solution

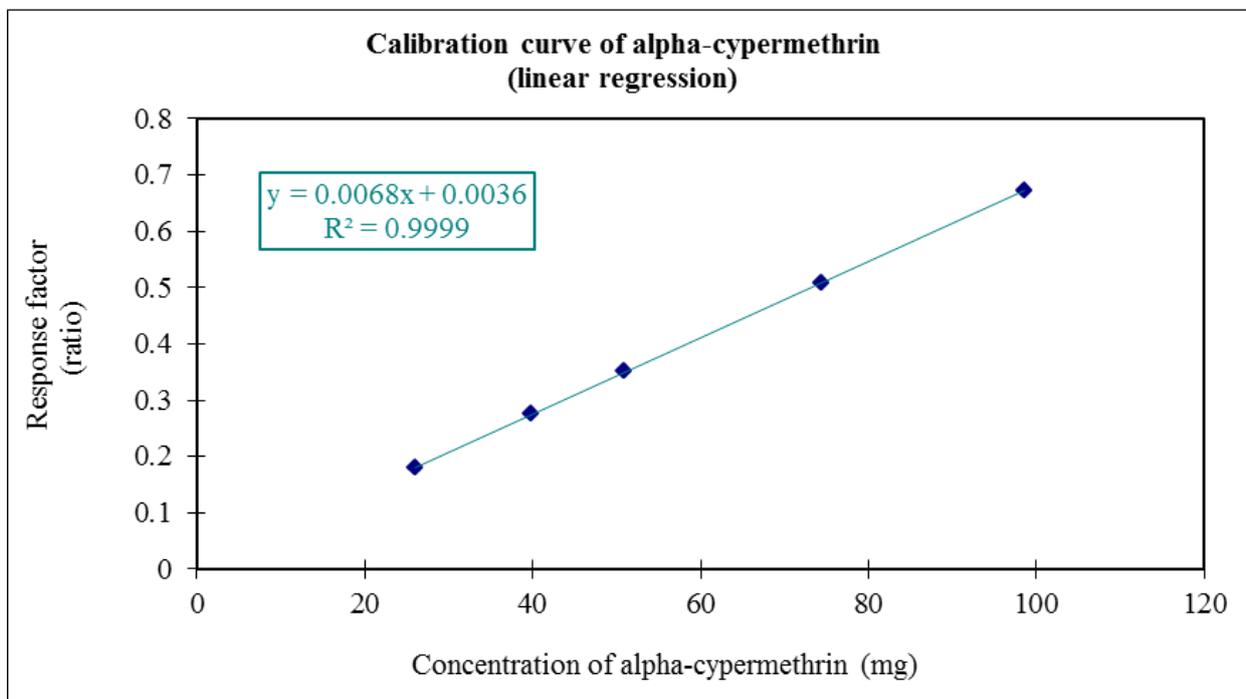
#### 4.2.2 Linearity of the chromatographic response

##### Alpha-cypermethrin

Code	Weight (mg)	H <sub>s</sub> (mAU*sec)	I <sub>r</sub> (mAU*sec)	Response ratio (H <sub>s</sub> /I <sub>r</sub> )
CA	26.1	96.59	538.31	0.18
CB	39.8	153.04	556.42	0.28
CC	50.8	161.09	459.20	0.35
CD	74.4	271.35	534.75	0.51
CE	98.5	352.22	523.08	0.67

<b>Slope</b>	0.0036
<b>Intercept</b>	0.0068
<b>CC</b>	$y = 0.0068x + 0.0036$

##### Calibration curve of alpha-cypermethrin by GC-FID

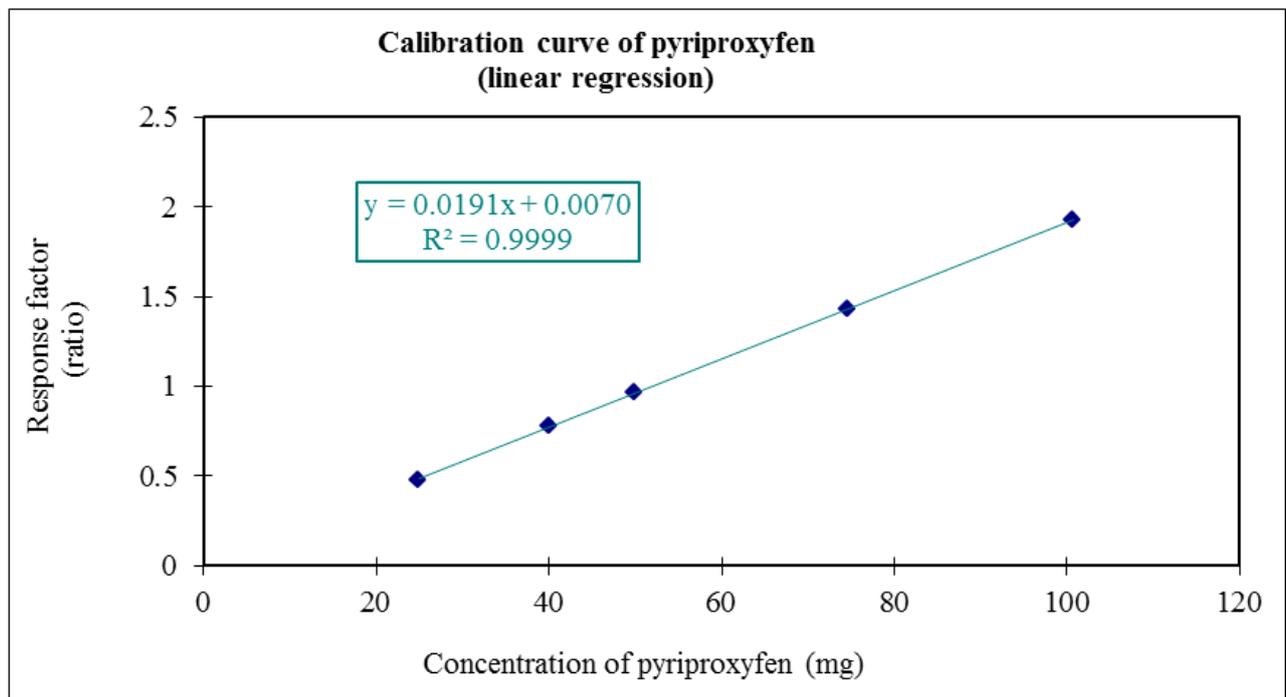


The linearity of the detector response meets the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

Pyriproxyfen

Code	Weight (mg)	H <sub>s</sub> (mAU*sec)	I <sub>r</sub> (mAU*sec)	Response ratio (H <sub>s</sub> /I <sub>r</sub> )
CA	25.0	3285.98	6898.47	0.48
CB	40.2	5417.28	6924.88	0.78
CC	50.1	6713.63	6962.43	0.96
CD	74.7	9828.96	6864.62	1.43
CE	100.8	13819.87	7167.70	1.93

<b>Slope</b>	0.0070
<b>Intercept</b>	0.0191
<b>CC</b>	$y = 0.0191x + 0.0070$

Calibration curve of pyriproxyfen by HPLC-DAD

The linearity of the detector response meets the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

#### 4.2.3 Repeatability and intra-laboratory reproducibility

Royal Guard, 120 denier

Royal Guard 120-D (Blue)							
Code	Pyriproxyfen (g/kg)			Code	Alpha-cypermethrin (g/kg)		
	Net No.1	Net No.2	Net No.3		Net No.1	Net No.2	Net No.3
D1S1	5.54	5.68	5.76	D1S1	5.84	5.94	5.95
D1S2	5.45	5.66	5.69	D1S2	5.89	6.00	6.02
D2S1	5.49	5.60	5.65	D2S1	5.53	5.72	5.73
D2S2	5.55	5.70	5.58	D2S2	5.86	5.95	5.90
<b>Mean</b>	<b>5.51</b>	<b>5.66</b>	<b>5.67</b>	<b>Mean</b>	<b>5.78</b>	<b>5.90</b>	<b>5.90</b>
<b>SD</b>	<b>0.05</b>	<b>0.05</b>	<b>0.07</b>	<b>SD</b>	<b>0.17</b>	<b>0.12</b>	<b>0.12</b>
<b>%RSD</b>	<b>0.83%</b>	<b>0.80%</b>	<b>1.31%</b>	<b>%RSD</b>	<b>2.93%</b>	<b>2.09%</b>	<b>2.08%</b>
<b>HL</b>	<b>4.38</b>	<b>4.36</b>	<b>4.36</b>	<b>HL</b>	<b>4.34</b>	<b>4.33</b>	<b>4.33</b>

The repeatability and intra-laboratory reproducibility meet the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

Royal Guard, 150 denier

Royal Guard 150-D (White)							
Code	Pyriproxyfen (g/kg)			Code	Alpha-cypermethrin (g/kg)		
	Net No.1	Net No.2	Net No.3		Net No.1	Net No.2	Net No.3
D1S1	5.61	5.53	5.47	D1S1	6.11	6.06	6.08
D1S2	5.54	5.45	5.54	D1S2	6.08	5.99	6.07
D2S1	5.65	5.48	5.49	D2S1	6.03	5.99	6.00
D2S2	5.64	5.50	5.47	D2S2	5.93	5.87	5.88
<b>Mean</b>	<b>5.61</b>	<b>5.49</b>	<b>5.49</b>	<b>Mean</b>	<b>6.04</b>	<b>5.98</b>	<b>6.01</b>
<b>SD</b>	<b>0.05</b>	<b>0.03</b>	<b>0.04</b>	<b>SD</b>	<b>0.08</b>	<b>0.08</b>	<b>0.09</b>
<b>%RSD</b>	<b>0.88%</b>	<b>0.62%</b>	<b>0.64%</b>	<b>%RSD</b>	<b>1.27%</b>	<b>1.37%</b>	<b>1.55%</b>
<b>HL</b>	<b>4.36</b>	<b>4.38</b>	<b>4.38</b>	<b>HL</b>	<b>4.32</b>	<b>4.32</b>	<b>4.32</b>

The repeatability and intra-laboratory reproducibility meet the CIPAC and EU requirements (Documents CIPAC 3807 and SANCO 3030/99).

## 5 Opinions, interpretations and advices\*\*

\*\* out of accreditation

## 6 Remark

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#### The Laboratory Manager

Name: Dr ir Olivier PIGEON

Signature:

Date: