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**PYRIPROXYFEN AND**

**ALPHA-CYPERMETHRIN**

**CIPAC/4887 (extension of CIPAC 715/TC/M/3) and CIPAC/5043 (extension of CIPAC 454/LN/M/3.2)**

**Method Extension For The Determination of Pyriproxyfen And Alpha-Cypermethrin In Long Lasting Insecticidal Net**

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**Method Extension of Existing CIPAC Methods for Pyriproxyfen And Alpha-Cypermethrin In Long Lasting Insecticidal Net - Royal Guard®**

**Disease Control Technologies / Clariant**

**BY**

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## 1. Introduction

The method CIPAC 715/TC/M/3 was extended to determine the pyriproxyfen without any modifications.

The method **CIPAC/4887** (extension of CIPAC 715/TC/M/3) was extended to determine the alpha-cypermethrin with minor modifications (CIPAC/5043).

## 2. Chemical Details of the Test Item

The test item was provided by the Sponsor DCT.  On completion of the study, any unused test item will be disposed of following the SOP of the testing laboratory.

Test Item   :   Alpha-cypermethrin and Pyriproxyfen incorporated long lasting insecticidal nets

I.     Royal Guard® - 120 Denier Spec. (Blue)

| Substance Name | CAS No. | Function | g/kg |
|---|---|---|---|
| Alpha-cypermethrin | 67375-30-8 | Insecticide | 6.25 |
| Pyriproxyfen | 95737-68-1 | Insecticide | 6.58 |

II.     Royal Guard® – 150 Denier Spec. (White)

| Substance Name | CAS No. | Function | g/kg |
|---|---|---|---|
| Alpha-cypermethrine | 67375-30-8 | Insecticide | 5.83 |
| Pyriproxyfen | 95737-68-1 | Insecticide | 5.54 |

IUPAC name

Alpha-cypermethrin   :   (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylate and (R)- α-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

Pyriproxyfen   :   4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether

Chemical abstract name

Alpha-cypermethrin   :   [1α(S*),3α]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2- dimethylcyclo propanecarboxylate

Pyriproxyfen   :   2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy]pyridine

Empirical formula

Alpha-cypermethrin   :   $C_{22}H_{19}Cl_2NO_3$

Pyriproxyfen   :   $C_{20}H_{19}NO_3$

Molecular weight

Alpha-cypermethrin           :           416.3

Pyriproxyfen                 :           321.4
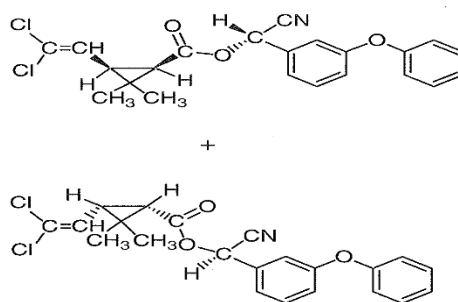
Batch No.                    :           120-D  & 150-D
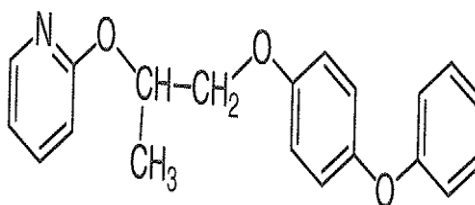
Insecticidal net specification :         120-D

(based on linear density)    :           150-D

Molecular structure

Alpha-cypermethrin           :



+



Pyriproxyfen                 :



Details of Supplier          :           **Clariant International Ltd.,**
                                         Rothausstrasse 61,
                                         CH-4132 Muttenz,
                                         SWITZERLAND

**Reference Item Details**

Reference item               :           Alpha-cypermethrin

Batch No                     :           AMT057E15

Validation Date              :           01.05.2014

Expiry Date                  :           30.04.2017

Purity                       :            99.25 %

Source                       :           Tagros Chemicals India limited, Chennai.

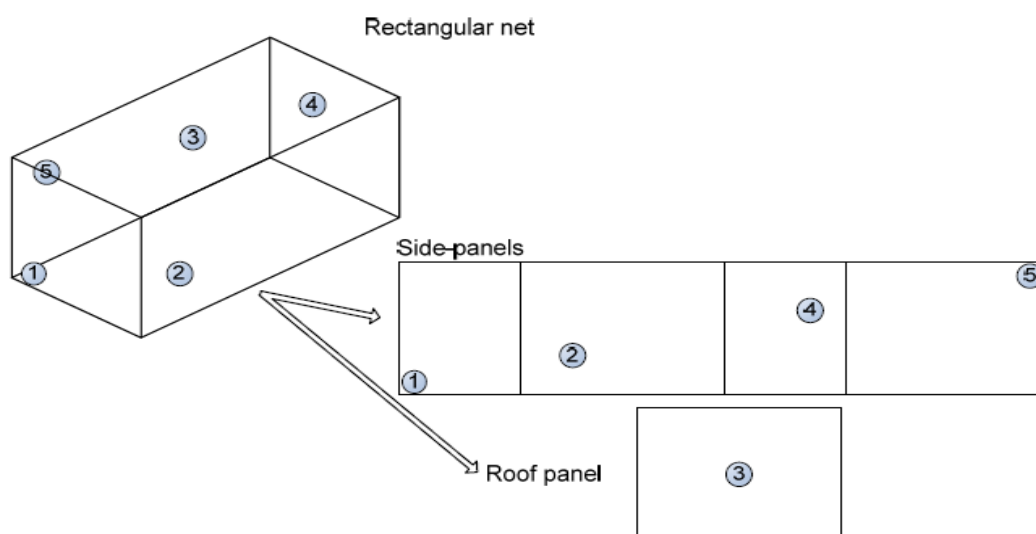|                     |   |                                      |
|---------------------|---|--------------------------------------|
| Storage temperature | : | <20°C                                |
| Reference item      | : | Pyriproxyfen                         |
| Batch No            | : | TCIRD-028-PPFN                       |
| Production Date     | : | November, 2015                       |
| Expiry Date         | : | October, 2017                        |
| Purity              | : | 99.02%                               |
| Source              | : | Tagros Chemicals India limited, Chennai. |
| Storage temperature | : | <25°C                                |

## 3. Objectives of the study

The objective of this study was to determine alpha-cypermethrin and pyriproxyfen content in the insecticide incorporated long lasting net Royal Guard®. The pyriproxyfen content in test item was determined by HPLC method with reference to CIPAC/4887 for Pyriproxyfen (June 2013) and CIPAC Handbook M, 715/TC/M/3 and the alpha-cypermethrin content was determined by GC-FID according to CIPAC 454/LN/M/3.2.

## 4. Experimental procedure

### 4.1 Test item sampling

Recommended positions from which 5 pieces of netting will be taken from alpha-cypermethrin and pyriproxyfen impregnated long lasting insecticidal net (LLIN) material and combined to form a representative sample.



5 pieces of 25 cm x 25 cm are cut from each side / roof of an entire net and pooled together and wrapped in an aluminium foil to form a laboratory sample representative of the entire net. This operation is performed 2 times at adjacent places in order to provide the 2 laboratories (IIBAT

and CRA-W) with equivalent samples. This operation is performed on 3 nets of Royal Guard®, 120 denier and 3 nets of Royal Guard®, 150 denier. Each laboratory then received 3 samples of Royal Guard®, 120 denier and 3 samples of Royal Guard®, 150 denier, each sample consisting of 5 pieces of25 cm x 25 cm pooled together.

## 4.2 Chemical analysis of samples at IIBAT and CRA-W

Each laboratory analyzed the samples following the IIBAT method presented at the 2016 CIPAC Technical Meeting (CIPAC/5043). Duplicate aliquots (fresh weights) on 2 different days were performed on the 3 samples of Royal Guard®, 120 denier and the 3 samples of Royal Guard®, 150 denier. The CRA-W sent their results to IIBAT in the form of a test report.

## 5. Method Description

### PYRIPROXYFEN AND ALPHA-CYPERMETHRIN LONG LASTING INSECTICIDAL NET METHOD EXTENSION OF CIPAC 715/TC/M/3 and CIPAC/4887

### 5.1 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 (CIPAC 715/TC/M/3). The same sample extracted in heptane is used for the determination of alpha-cypermethrin by GC-FID (CIPAC 454/LN/M/3.2).

### 5.2 Reagents

- Heptane
- 2-propanol
- Tetrahydrofuran
- Acetonitrile HPLC Grade
- Water HPLC grade or MilliQ® grade water
- Pyriproxyfen and Alpha-cypermethrin reference analytical standards of known purity, stored in refrigerator.
- Di-cyclohexyl phthalate internal standard, must not show any peaks with the same retention time as pyriproxyfen and alpha-cypermethrin
- Citric acid 10% solution. Dissolve citric acid (50 g) in tetrahydrofuran (500 mL)

### 5.3 Internal Standard Solution

Dissolve dicyclohexyl phthalate 5.0 g in 200 mL Volume of 2-propanol and use for the preparation of all calibration and sample solutions.

## 5.4 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 (solution $C_A$, $C_B$, $C_C$, $C_D$ and $C_E$) respectively. Prepare five calibration solutions comprising approximately 0.5, 0.8, 1.0, 1.5, and 2.0 times that of calibration solution.

## 5.5 Calibration solutions for alpha-cypermethrin

Weigh about 25, 40, 50, 75 and 100 mg (to the nearest 0.1mg) of Alpha-cypermethrin analytical standard in five 50 mL volumetric flasks. Add by pipette to each flask 5 mL internal standard solution,2 mL of citric acid solution and fill to the mark with heptane (solution $C_A$, $C_B$, $C_C$, $C_D$ and $C_E$) respectively. Prepare five calibration solutions comprising approximately 0.5, 0.8, 1.0, 1.5, and 2.0 times that of calibration solution.

## 5.6 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 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 and 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 $S_B$).

## 5.7 Sample analysis

The solution were injected in the sequence CC, S1R1, S1R2, CC, S2R1, S2R2, CC…. etc.,

## 5.8 Analytical Conditions

### Pyriproxyfen

| Lab | Liquid chromatograph integrator | Column | Mobile phase | Flow rate (mL/ min) | Column temp (°C) | Volume injected (µL) | Detector Wave length |
|---|---|---|---|---|---|---|---|
| Lab-1 CRA-W | Agilent 1200 Series HPLC-DAD detector | Phenomenex Luna C18-2, 250 mm x 4.6 mm i.d x 5 µm | acetonitrile – water, 70/30 (v/v) | 1 | 40 | 10 | 254nm |
| Lab-2 IIBAT | Agilent 1290 Series HPLC-DAD detector | Nucleosil C18 (5µm,) or C18 column 250 mm x 4.6 mm i.d x 5 µm | acetonitrile – water, 70/30 (v/v) | 1 | 40 | 10 | 254nm |

**Alphacypermethrin**

| Lab | Gas chromatograph integrator | Column | Carrier gas | Make-up gas Flow rate (mL/min) | Temperatures (°C) | Volume injected µL |
|---|---|---|---|---|---|---|
| Lab-1 CRA-W | Agilent 6890Series GC-FID | DB-1 capillary fused silica coated with 100% methyl siloxane 30 m x 0.25 mm i.d x 0.25 µm | 0.8 ml/min Helium as a carrier gas | 30 (helium) | Column Oven-235°C Injector-260°C Detector-300°C | 1 |
| Lab-2 IIBAT | Shimadzu GC-2010 series GC-FID | DB-1 capillary Column 30 m x 0.25 mm i.d x 0.25µm | 0.8 ml/min Helium as a carrier gas | 40 (helium) | Column Oven-230°C Injector-260°C Detector-300°C | 1 |

**Deviations from the Analytical Method**

**No deviations.**

## Pyriproxyfen

### (a) 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 in CIPAC 715. The internal standard dicyclohexyl phthalate was used while preparing the calibration solution of pyriproxyfen.

### (b) 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%.

### (c) 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.

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

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

Where:

| | | |
|---|---|---|
| *fi* | = | Individual response factor |
| *f* | = | Mean response factor |
| *Hs* | = | Peak area of active ingredient in calibration solution |
| *Hw* | = | Peak area of active ingredient in sample solution |
| *Ir* | = | Peak area of internal standard in the calibration solution |
| *Iq* | = | 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) |

**Alpha-cypermethrin**

*(a) Linearity check.*

Before conducting the analysis check the linearity of the detector response by injecting 1 µl portions of five calibration solutions of alpha-cypermethrin concentrations as described in CIPAC 454. The internal standard dicyclohexyl phthalate was used while preparing the calibration solution of alpha-cypermethrin.

*(b) 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%.

*(c) 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.

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

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

Where:

| | | |
|---|---|---|
| *fi* | = | Individual response factor |
| *f* | = | Mean response factor |
| *Hs* | = | Peak area of active ingredient in calibration solution |
| *Hw* | = | Peak area of active ingredient in sample solution |
| *Ir* | = | Peak area of internal standard in the calibration solution |
| *Iq* | = | 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) |

## 6. Method Assessment

According to the CIPAC method extension guideline, the method extension of the CIPAC 715/TC/M/3 and CIPAC 4887 for pyriproxyfen and alpha-cypermethrin LN is investigated. In addition to specificity and repeatability tests, accuracy test was conducted to confirm that pyriproxyfen was determined accurately in the presence of alpha-cypermethrin.

The sample subjected to this assessment was Royal Guard® – 120D & 150 D.

The 120-D LLIN has nominal contents of pyriproxyfen 5.5 g/kg and alpha-cypermethrin5.5 g/kg.

The 150-D LLIN has nominal content of pyriproxyfen 5.0 g/kgand alpha-cypermethrin5.0 g/kg.

## 6.1 Modification of method

Method also demonstrates the use of heptane for the determination of alpha-cypermethrin using the same internal standard dicyclohexyl phthalate.

These modifications are considered to be minor modifications.

## 6.2 Check of the acceptability range

The pyriproxyfen content and alpha-cypermethrinin LN should be within the acceptability range of the existing CIPAC methods.

## 6.3 Specificity

The instrument response of solvents (n-Heptane, 2-propanol, and acetonitrile and milliQwater) was compared with response of reference analytical standards and extract of long lasting mosquito net. The results reveal either the solvents or the components of long lasting insecticidal net has no significant interference.

## 6.4 Linearity of the Detector Response

**Pyriproxyfen:**

The instrument response was found to be linear against the concentration of pyriproxyfen. Five linear concentration solutions representing including 0.5 mg/mL, 0.80 mg/mL, 1.0 mg/mL, 1.5 mg/mL and 2.0 mg/mL concentration of pyriproxyfen long lasting insecticidal content in net were prepared for linearity determination.

**Alpha-cypermethrin:**

The instrument response was found to be linear against the concentration of alpha-cypermethrin. Five linear concentration solutions representing including 0.5 mg/mL, 0.80 mg/mL, 1.0 mg/mL, 1.5 mg/mL and 2.0 mg/mL concentration of alpha-cypermethrin long lasting insecticidal content in net were prepared for linearity determination.

The results reveal that the instrument response factor was linear against the concentration of pyriproxyfen and alpha-cypermethrin respectively which was substantiated by the correlation co-efficient 1.0000 and 0.9999 for pyriproxyfen and alpha-cypermethrin respectively. The results are summarized as follows.
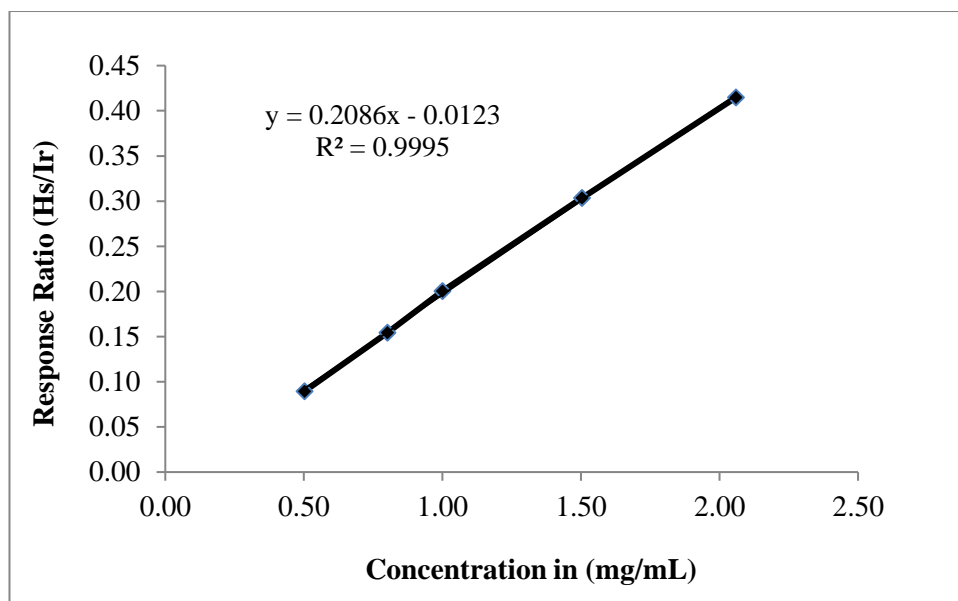
## TABLE 1    CALIBRATION DATA ON ALPHA – CYPERMETHRIN

|  | Lab-1 CRA-W | Lab-2 IIBAT |
|---|---|---|
| Range | 26.1-98.5 (mg) | 25.09-102.97 (mg) |
| Slope | 0.0036 | 0.2086 |
| Intercept | 0.0068 | -0.01235 |
| Correlation Coefficient (CC) | 1.0000 | 0.9997 |

## TABLE 2 CALIBRATION DATA ON PYRIPROXYFEN

|  | Lab-1 CRA-W | Lab-2 IIBAT |
|---|---|---|
| Range | 25.0-100.8 (mg) | 25.11-100.75 (mg) |
| Slope | 0.0070 | 0.8621 |
| Intercept | 0.0191 | 0.15245 |
| Correlation Coefficient (CC) | 0.9999 | 0.9996 |

**FIGURE 1    CALIBRATION CURVE OF ALPHA-CYPERMETHRIN**



$y = 0.2086x - 0.0123$
$R^2 = 0.9995$

**CALIBRATION CURVE OF PYRIPROXYFEN**



$y = 0.8621x + 0.1524$
$R^2 = 0.9993$

## 6.5 Repeatability and intra-laboratory reproducibility

The actives, *Viz.,* pyriproxyfen and alpha-cypermethrin in both the long lasting mosquito insecticidal net has been determined and the quantified values are summarized as follows.

### Quantification of Day 1 and Day 2 Royal Guard® 120-D, LN – Lab-1 CRA-W

| Lab-1 CRA-W | | | | | | |
|---|---|---|---|---|---|---|
| | Pyriproxyfen (g/kg) | | | Alpha- Cypermethrin(g/kg) | | |
| Day 1 | Net No.1 | Net No.2 | Net No.3 | Net No.1 | Net No.2 | Net No.3 |
| R1 | 5.54 | 5.68 | 5.76 | 5.84 | 5.94 | 5.95 |
| R2 | 5.45 | 5.66 | 5.69 | 5.89 | 6.00 | 6.02 |
| Day 2 | | | | | | |
| R1 | 5.49 | 5.60 | 5.65 | 5.53 | 5.72 | 5.73 |
| R2 | 5.55 | 5.70 | 5.58 | 5.86 | 5.95 | 5.90 |
| Mean | 5.51 | 5.66 | 5.67 | 5.78 | 5.90 | 5.90 |
| SD | 0.05 | 0.05 | 0.07 | 0.17 | 0.12 | 0.12 |
| % RSD | 0.83 | 0.80 | 1.31 | 2.93 | 2.09 | 2.08 |
| HL | 4.38 | 4.36 | 4.36 | 4.34 | 4.33 | 4.33 |

# Quantification of Day 1 and Day 2 Royal Guard® 120-D, LN – Lab-2 IIBAT

| Lab-2 IIBAT | | | | | | |
|---|---|---|---|---|---|---|
| | Pyriproxyfen (g/kg) | | | Alpha- Cypermethrin(g/kg) | | |
| Day 1 | Net No.1 | Net No.2 | Net No.3 | Net No.1 | Net No.2 | Net No.3 |
| R1 | 6.42 | 6.40 | 6.37 | 6.22 | 6.21 | 6.20 |
| R2 | 6.34 | 6.40 | 6.44 | 6.20 | 6.23 | 6.27 |
| Day 2 | | | | | | |
| R1 | 6.42 | 6.41 | 6.40 | 6.25 | 6.30 | 6.23 |
| R2 | 6.41 | 6.46 | 6.39 | 6.23 | 6.21 | 6.19 |
| Mean | 6.40 | 6.42 | 6.40 | 6.23 | 6.24 | 6.22 |
| SD | 0.039 | 0.029 | 0.029 | 0.021 | 0.043 | 0.036 |
| % RSD | 0.60 | 0.45 | 0.46 | 0.33 | 0.68 | 0.58 |
| HL | 4.28 | 4.28 | 4.28 | 4.30 | 4.30 | 4.30 |

# Quantification of Day 1 and Day 2 Royal Guard® 150-D, LN – Lab-1 CRA-W

| Lab-1 CRA-W | | | | | | |
|---|---|---|---|---|---|---|
| | Pyriproxyfen (g/kg) | | | Alpha- Cypermethrin(g/kg) | | |
| Day 1 | Net No.1 | Net No.2 | Net No.3 | Net No.1 | Net No.2 | Net No.3 |
| R1 | 5.61 | 5.53 | 5.47 | 6.11 | 6.06 | 6.08 |
| R2 | 5.54 | 5.45 | 5.54 | 6.08 | 5.99 | 6.07 |
| Day 2 | | | | | | |
| R1 | 5.65 | 5.48 | 5.49 | 6.03 | 5.99 | 6.00 |
| R2 | 5.64 | 5.50 | 5.47 | 5.93 | 5.87 | 5.88 |
| Mean | 5.61 | 5.49 | 5.49 | 6.04 | 5.98 | 6.01 |
| SD | 0.05 | 0.03 | 0.04 | 0.08 | 0.08 | 0.09 |
| % RSD | 0.88 | 0.62 | 0.64 | 1.27 | 1.37 | 1.55 |
| HL | 4.36 | 4.38 | 4.38 | 4.32 | 4.32 | 4.32 |

# Quantification of Day 1 and Day 2 Royal Guard® 150-D, LN – Lab-2 IIBAT

| Lab-2 IIBAT | | | | | | |
|---|---|---|---|---|---|---|
| | Pyriproxyfen (g/kg) | | | Alpha- Cypermethrin(g/kg) | | |
| Day 1 | Net No.1 | Net No.2 | Net No.3 | Net No.1 | Net No.2 | Net No.3 |
| R1 | 5.35 | 5.34 | 5.45 | 5.75 | 5.68 | 5.70 |
| R2 | 5.48 | 5.44 | 5.35 | 5.76 | 5.53 | 5.64 |
| Day 2 | | | | | | |
| R1 | 5.32 | 5.30 | 5.36 | 5.60 | 5.61 | 5.59 |
| R2 | 5.40 | 5.39 | 5.30 | 5.67 | 5.62 | 5.76 |
| Mean | 5.39 | 5.37 | 5.37 | 5.70 | 5.61 | 5.67 |
| SD | 0.070 | 0.061 | 0.062 | 0.075 | 0.062 | 0.074 |
| % RSD | 1.30 | 1.13 | 1.16 | 1.32 | 1.10 | 1.30 |
| HL | 4.39 | 4.39 | 4.39 | 4.36 | 4.36 | 4.36 |

## 7. Conclusion

In order to apply the CIPAC 715/TC/M/3 to pyriproxyfen and alpha-cypermethrin LN combination, the thermal extraction step using heptane was used.

In order to apply the CIPAC 4887 to alpha-cypermethrin in LN the LN extract of 715 was used to determine alpha-cypermethrin. This carry forward the internal standard from 715 (Dicyclohyxylphthalate) instead of Dioctylphthalate, hence the alpha-cypermethrin calibration solution was prepared using Dicyclohyxylphthalate
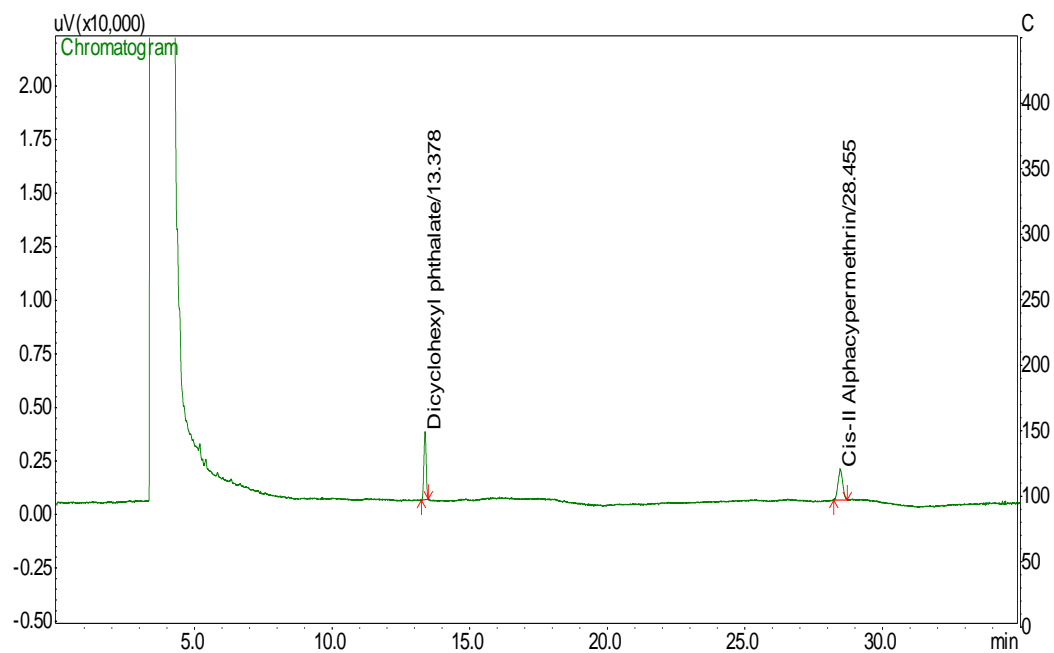
These modifications are considered to be minor modifications and the proposed method shall be accepted.
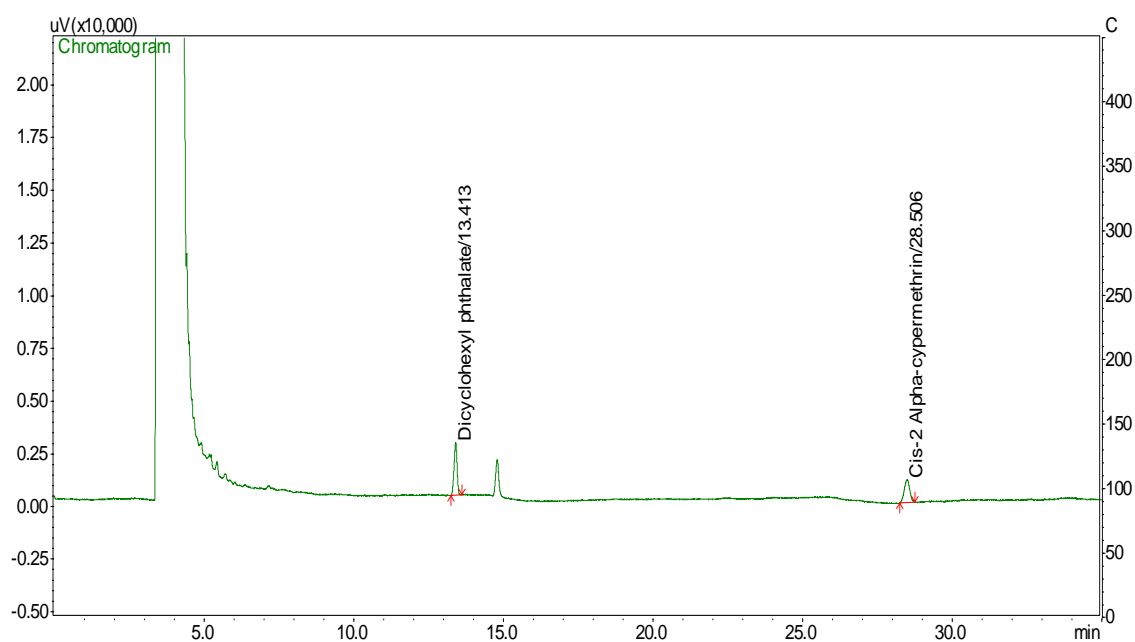
Annexure: 1 Test report RE/17/U10/24406 from CRA-W

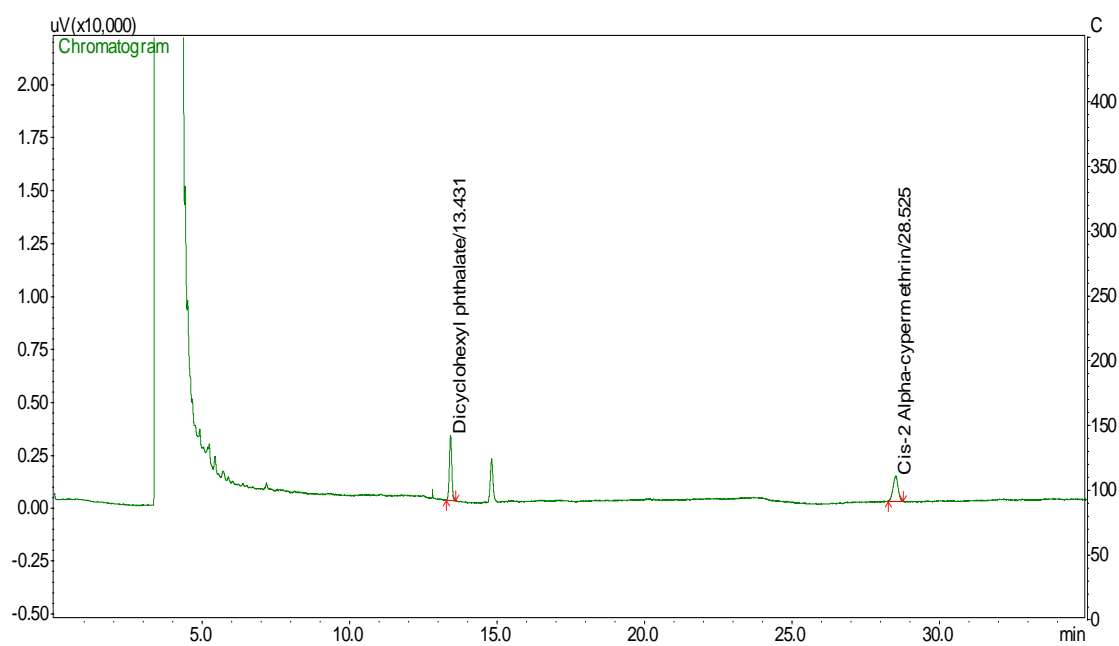# REPRESENTATIVE CHROMATOGRAM OF ALPHACYPERMETHRIN - GC-FID (ZOOMED)



# REPRESENTATIVE CHROMATOGRAM OF CALIBRATION SOLUTION OF ALPHACYPERMETHRIN - GC-FID
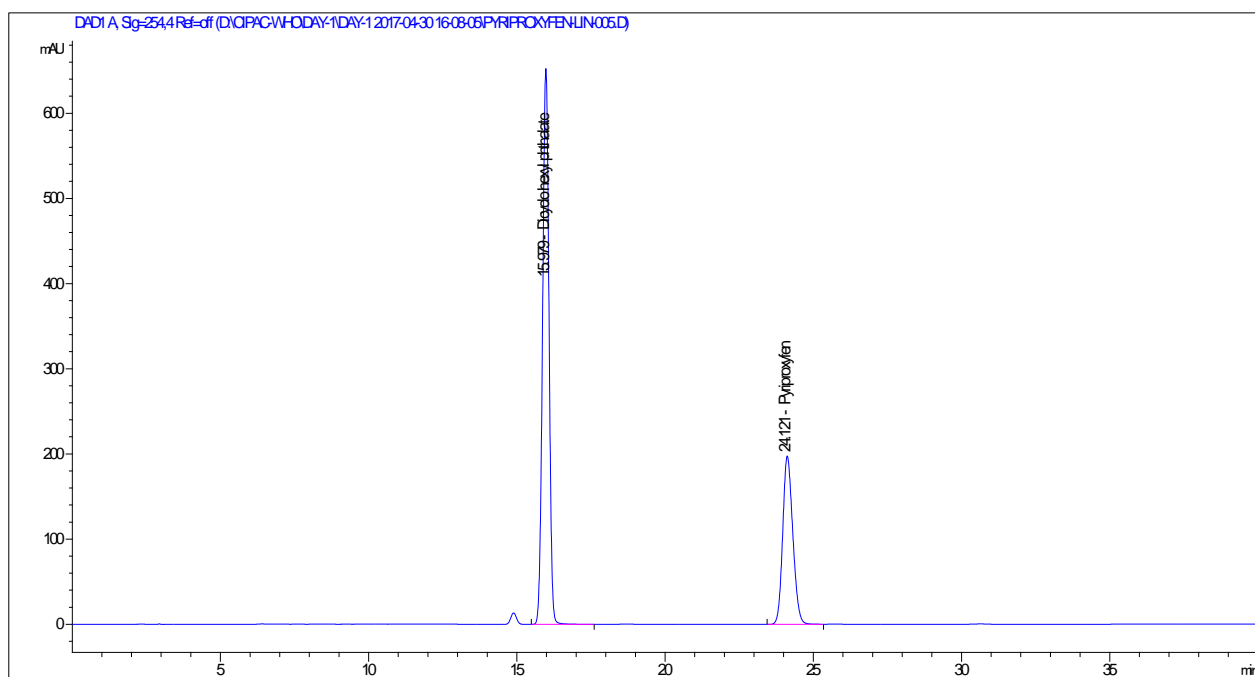
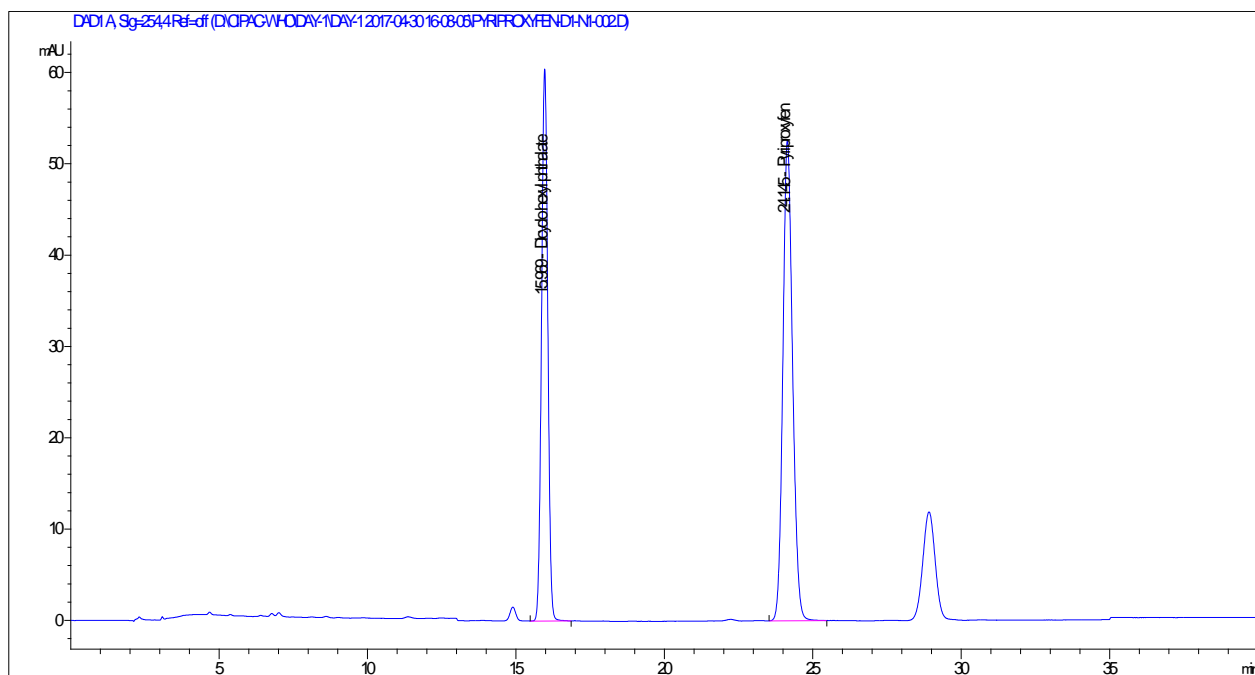# REPRESENTATIVE CHROMATOGRAM OF LN 120-D - GC-FID



# REPRESENTATIVE CHROMATOGRAM OF LN 150-D - GC-FID

# REPRESENTATIVE CHROMATOGRAM OF CALIBRATION SOLUTION OF PYRIPROXYFEN - HPLC



# REPRESENTATIVE CHROMATOGRAM OF LN 120-D - HPLC

# REPRESENTATIVE CHROMATOGRAM OF LN 150-D - HPLC



DAD1 A, Sig=254,4 Ref=off (D:\CIPAC\WHO\150-DAY-1\DAY-1 2017-05-03 09-44-45\PYRIPROXYFEN D1-N1-003.D)