

**STUDIES ON THE WASH RESISTANCE INDEX OF  
LONG LASTING INSECTICIDE NET (DURANET PLUS)  
CONTAINING ALPHA-CYPERMETHRIN 0.58 % W/W AND  
PIPERONYL BUTOXIDE 0.2 % W/W**

**STUDY NUMBER**

042015200

**ADDENDUM REPORT**

**CONDUCTED BY**

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25/05/2016

**SPONSORED BY**

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**CERTIFICATE**

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CYPERMETHRIN 0.58 % W/W AND PIPERONYL BUTOXIDE 0.2 % W/W**

Study No. : 042015200

conducted by

Department of Analytical Chemistry

International Institute of Biotechnology And Toxicology

Padappal-601 301, Tamil Nadu, India

is

true, accurate and a faithful record of the results obtained. We, the undersigned hereby certify the authenticity of the same.

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**Date: 25/05/2016**

## **STUDIES ON THE WASH RESISTANCE INDEX OF LONG LASTING INSECTICIDE NET (DURANET PLUS) CONTAINING ALPHA- CYPERMETHRIN 0.58 % W/W AND PIPERONYL BUTOXIDE 0.2 % W/W**

### **1.0 SUMMARY**

Duranet plus mosquito net containing incorporated Alpha-cypermethrin 0.58 % w/w and Piperonyl butoxide 0.2 % w/w were supplied by Shobikaa Impex Private Limited (India), Karur. The studies on the wash resistance index of long lasting insecticide net (duranet plus) containing alpha-cypermethrin 0.58 % w/w and piperonyl butoxide 0.2 % w/w was conducted following CIPAC MT 195 by the Department of Analytical Chemistry, International Institute of Biotechnology And Toxicology (IIBAT), Padappai - 601 301, Kancheepuram District, Tamil Nadu, India.

The results obtained in the study conducted at  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  indicates:

The retention index of Alpha-cypermethrin after 4<sup>th</sup> wash 99.54% and

The retention index of piperonyl butoxide after 4<sup>th</sup> wash was 93.56%.

Thus, from the above experiment it can be concluded that the compound is stable and the actives are within the limits fulfilling the requirements of 454/LN/M/3.2 and 33/LN/M/3.0.

## 2.0 GENERAL INFORMATION

1. Name and address of the sponsor : Shobikaa Impex Private Limited (India)  
34, Sannathi Street,  
Vennaiimalai (Po)  
Karur - 639006
2. Name and address of the research institute : International Institute of Biotechnology  
And Toxicology (IIBAT),  
Padappai - 601 301, Kancheepuram  
District, Tamil Nadu, India.
3. Test facility : Department of Analytical Chemistry, IIBAT.
4. Nature of the study : Retention index of alpha cypermethrin  
and piperonyl butoxide in Duranet plus  
following CIPAC MT 195

## 2.1 TEST ITEM DETAILS

1. Common name : LLIN Incorporated with  
Alpha-cypermethrin 0.58% w/w and  
Piperonyl Butoxide 0.2% w/w
2. Trade Name : Duranet plus
3. Size of Net : 190 (W) x 180 (L) x 150 (H) in cm
4. Batch Number : S5W0415
5. Appearance : White colour net, packed in Plain  
Transparent Clear bag (pouch)

### 3.0 CHEMICAL DETAILS OF PURE COMPOUND

#### ALPHA –CYPERMETHRIN

1. IUPAC name : A racemate comprising  
(*S*)- $\alpha$ -cyano-3-phenoxybenzyl  
(1*R*,3*R*)-3-(2,2-dichlorovinyl)-  
2,2-dimethylcyclopropanecarboxylate and  
(*R*)-  $\alpha$ -cyano-3-phenoxybenzyl (1*S*,3*S*)-3-(2,2-  
dichlorovinyl)-2,2-  
dimethylcyclopropanecarboxylate
2. Chemical abstract name : [1 $\alpha$ (*S*<sup>\*</sup>),3 $\alpha$ ]-( $\pm$ )-cyano(3-phenoxyphenyl)  
methyl 3-(2,2-dichloroethenyl)-2,2-  
dimethylcyclopropanecarboxylate
3. CAS RN : [67375-30-8]
4. Empirical formula : C<sub>22</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>3</sub>
5. Molecular weight : 416.3

#### PIPERONYL BUTOXIDE

1. IUPAC name : 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-  
1,3-benzodioxole;2-(2-butoxyethoxy)ethyl 6-  
propylpiperonyl ether
2. Chemical abstract name : 5-[[2-(2-butoxyethoxy)ethoxy]methyl]-6-  
propyl-1,3-benzodioxole
3. CAS RN : [51-03-6]
4. Empirical formula : C<sub>19</sub>H<sub>30</sub>O<sub>5</sub>
5. Molecular weight : 338.4

### 3.1 TEST METHODOLOGY

1. Active ingredient (Alpha-cypermethrin) : By GC-FID method  
CIPAC Volume-M, 454/LN/M/3.2
2. Active ingredient (Piperonyl Butoxide) : By GC-FID method  
CIPAC Volume-N, 33/LN/M/3.0
3. Retention index : CIPAC MT 195

#### **4.0 METHODOLOGY FOR ACTIVE INGREDIENT CONTENT (ALPHA – CYPERMETHRIN) AND PIPERONYL BUTOXIDE**

##### **Outline of the method**

Suitable pieces of the net are subjected to successive washing-rinsing-heating cycles and the total active ingredient content is determined in washed samples after a number of washings. The wash resistance index is determined by the decrease of the total active ingredient content after several cycles using the appropriate analytical method following **CIPAC MT 195**.

##### **Reagents**

CIPAC washing agent (As per Note 1 of **MT 195**)

Deionized water

##### **Apparatus**

Thermostatically controlled Oven ( $\pm 0.5^{\circ}\text{C}$ )

Water bath capable of maintaining a specified temperature ( $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and large enough to allow six 1 L capped bottles to be immersed in an upright position in the water to the 500 ml mark

1 L screw capped glass bottles conforming to ISO 4796 and DIN 168 (outside diameter 101 mm, height 225 mm)

Glass bottles 100 mL fitted with screw-caps and fitted with polyethylene inserts.

Scissors

Tweezers

Stopwatch

Refrigerator



**Procedure****a) Sampling**

Cut 6 pieces of 25 × 25 cm from the net or netting. Subject three of the pieces to 4 washing-rinsing-heating cycles as described below in paragraphs b-c-d. Analyse the remaining 3 pieces for their content of active ingredient without any washing.

**(b) Washing**

Add 2.5 ml of the stock solution of the CIPAC washing agent (as per Note 1 of MT 195) to 500 ml of deionised water at 30°C ± 2°C in a 1 L glass bottle. Insert a piece of net and cap the bottle. Invert the bottle 10 times (180°). Place the bottle in the water bath in an upright position free from vibration (30°C ± 2°C) and not under direct sunlight. After 10 minutes, remove the net sample using tweezers, and remove any remaining adherent drops of wash fluid by gentle shaking.

**(c) Rinsing**

Insert the washed net sample into a 1 L glass bottle containing 500 ml of deionised water at 30°C ± 2°C, cap and invert the bottle 10 times (180°). Place the bottle in the water bath in an upright position free from vibration and not under direct sunlight. After 10 minutes, remove the net sample using tweezers, replace the deionised water, insert the sample into the 1 L bottle and repeat this rinsing step once more.

**(d) Heating**

Using tweezers pull out the net sample from rinsing fluid and carefully remove any remaining adherent water drops by gentle shaking. Allow the sample to dry for 30 minutes at room temperature on a line and keep protected from direct sunlight. Fold the sample carefully once or twice in each direction, place it loosely rolled in a glass bottle,

cap and store it in an oven at  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 22 hours  $\pm$  2 hours before starting the next washing cycle. Repeat the washing-rinsing-heating process (b-c-d) 3 more times. At the end each of the 3 pieces of net has been washed 4 times.

**(e) Assay**

Analysed each of the 6 net samples using the appropriate CIPAC method for determination of total active ingredient content (alphacypermethrin following 454/LN/M/3.2 and piperonyl butoxide 33/LN/M/3.0). Express results as g active ingredient per kg net material.

Unwashed samples and wash samples are analysed simultaneously to reduce the analytical error.

**(f) Calculation**

Calculate the wash resistance index, w, after the 4 washing-rinsing-heating cycles using the following equation (Following the equation of CIPAC MT 195):

$$w = 100 \times 4\sqrt{(t_4/t_0)}$$

where:

t<sub>4</sub> = total active ingredient content (in g/kg) after 4 washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t<sub>0</sub> = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces

w = wash resistance index, expressed as percentage

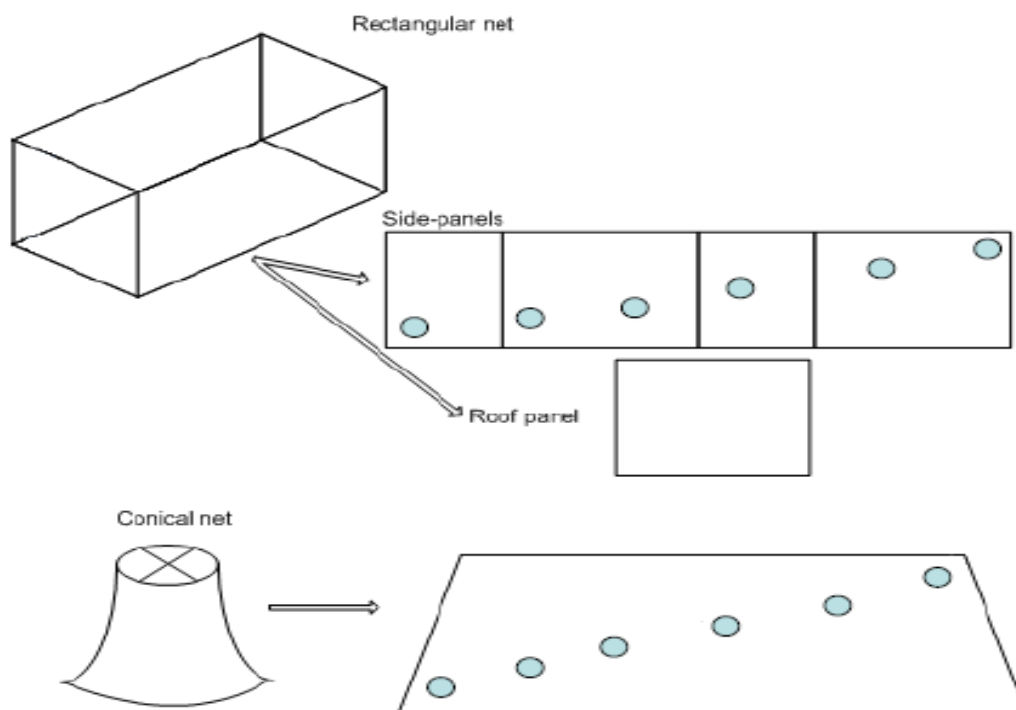
**(g) Reporting**

Report the wash resistance index to the nearest 1 % and specify the following parameters:

- temperature at which the heating procedure was performed if it was deviated from the standard ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ )
- assay method used

**Note 1** The stock solution of the CIPAC washing agent is prepared as follows: The bottle of polyoxyethylene glycol (25) monostearate (CAS number 9004-99-3 or 37231-60-0) is heated to about  $50^{\circ}\text{C}$  to melt and to reduce its viscosity. The bottle is turned through 180 degrees a few times to ensure homogeneity. Into a suitable 100 mL glass flask containing 80 ml of deionised water weight 12.0 g of sodium oleate (CAS number 143-19-1) plus 8.0 g of polyoxyethylene glycol (25) monostearate. Heat this mixture at about  $50^{\circ}\text{C}$  turning through 180 degrees frequently or stirring by a magnetic stirrer until the mixture becomes clear and homogeneous. This CIPAC washing agent can be used for up to 4 weeks if the flask is kept sealed in the dark in a refrigerator.

**Figure The positions from which 6 pieces of netting should be taken from a finished LN to form a representative sample**



## **5.0 METHODOLOGY FOR ACTIVE INGREDIENT CONTENT (ALPHA –CYPERMETHRIN) ANALYSIS FOLLOWING CIPAC VOLUME – M, 454/LN/M/3.2**

### **Outline of the method**

Alpha-cypermethrin content in the long lasting insecticide net sample (Duranet plus) was determined by GC-FID using CIPAC Volume – M, 454/LN/M/3.2 as reference method. The identity of the active ingredient was established by comparison with the equivalent authentic standard.

### **Preparation of 10% Citric acid solution**

Weighed exactly 50.0215 g of citric acid and transferred quantitatively in to 500 mL volumetric flask using tetrahydrofuran. The solution was mixed well and was made up to the volume mark using tetrahydrofuran. The solution was coded as “Citric acid 10% solution”.

### **Preparation of Internal standard solution**

Weighed exactly 50.21 mg of dioctylphthalate, internal standard in a tare, glass weighing bottle and the same was transferred quantitatively in to 1000 mL volumetric flask using tetrahydrofuran. The solution was mixed well and was made up to the volume mark using tetrahydrofuran. The solution was coded as “IS Solution”

### **Preparation of calibration solution**

Weighed exactly 126.5 mg alpha-cypermethrin reference standard in to a tare, glass weighing bottle and the same was transferred quantitatively in to a 1000 mL volumetric flask using Xylene as solvent. The solution was mixed well and was made up to the volume mark using Xylene. The solution was coded as “Alpha-cypermethrin stock”.

Accurately transferred using graduated pipette 40 mL, 60 mL, and 80 mL of “Alpha-cypermethrin stock” solution in to three separate 100 mL volumetric flasks. The flasks were made up to the volume mark using Xylene. The solutions were coded as C1, C2 and C3 respectively.

Accurately transferred 45 mL using graduated pipette, of each solution of *Viz.*, C1, C2 and C3 in to three separate glass stoppered bottles of capacity 150 mL. Additionally 50 mL of internal standard “IS Solution” and 5 mL of “Citric acid 10% solution” was dispensed using graduated pipette in to each flask. The solutions were mixed well. The flasks were coded as CA, CB and CC respectively.

## 5.1 CHROMATOGRAPHIC SEPARATION PARAMETERS (ALPHA-CYPERMETHRIN)

Instrument	-	Shimadzu Gas Chromatograph GC-2010 equipped with auto injector, Flame ionization detector and interfaced with GC solution software.
Detector	-	FID
Column	-	DB-1 Capillary Column (30m length x 0.25 mm I.D. x 0.25 $\mu$ film thickness).
Gas flow rate		
Column flow (N <sub>2</sub> )	-	0.66 ml/min
Hydrogen (H <sub>2</sub> )	-	40 ml/min
Air	-	400 ml/min
Make up (N <sub>2</sub> )	-	30 mL/min
Temperature		
Oven	-	227 °C
Injector	-	260 °C
Detector	-	300 °C
Volume injected	-	1 $\mu$ l
Injection Mode	-	Split
Split ratio	-	50: 1
Retention time (approximate)		
Diocetylphthalate ( <i>Internal standard</i> )	-	14.5 min
Alpha-Cypermethrin	-	27.5 min

## 5.2 DETERMINATION OF ALPHA-CYPERMETHRIN ( ASSAY - 454/LN/M)

### Preparation of sample solution

The Duranet plus long lasting insecticide net incorporated with alpha-cypermethrin and piperonyl butoxide was weighed appropriately such a way that the quantization fits to linear range of calibration, as per the sampling scheme mentioned in CIPAC Volume – M, 454/LN/M. The weighed samples were transferred to three separate 250 mL ground glass round bottomed flask. Each of the flask was added with 90 mL Xylene and 10 mL of “Citric acid 10% solution”. The flasks were coded as S1, S2 and S3 with prefix label as Duranet plus.

The flasks were connected to reflux condenser and refluxed for 30 min. The solutions in each flask were cooled to room temperature. This was followed by addition of 100 mL internal standard solution “IS Solution” to each flask. The solution was filtered through 45 µm filter and a small aliquot was transferred to GC Vial.

### Linearity Determination

The calibration solutions CA, CB and CC were injected in duplicate in to GC system equipped with FID. The detector response was recorded and a calibration curve was constructed by plotting the alpha-cypermethrin to internal standard peak area ratio versus the mass of alpha-cypermethrin in the calibration solutions.

### Sample Determination

The calibration solution was injected in duplicates followed by sample solutions in the sequence CB, CB, S1, S2, S3, CB, CB. The detector response was recorded and the alpha-cypermethrin content in long lasting insecticide net (Duranet plus) was calculated.

Mean response factor of calibration solution

$$f = \frac{I_r \times s \times P \times v}{H_s}$$

$$\text{Alpha-cypermethrin content (g/kg)} = \frac{H_w \times f \times 2}{I_q \times w}$$

$$\text{Alpha-cypermethrin content (\% w/w)} = \frac{H_w \times f \times 2 \times 100}{I_q \times w \times 1000}$$

Where,

H <sub>s</sub>	Peak area of Alpha-cypermethrin reference standard in calibration solution, (μV*Sec)
I <sub>r</sub>	Peak area of internal standard in calibration solution, (μV*Sec)
H <sub>w</sub>	Peak area of Alpha-cypermethrin in sample solution, (μV*Sec)
I <sub>q</sub>	Peak area of internal standard in sample solution, (μV*Sec)
w	Weight of net sample, (mg)
f	Response factor
P	Purity of Alpha-cypermethrin reference standard (mg)
s	Weight of Alpha-cypermethrin reference standard (mg)
v	Dilution factor 0.027



## **6.0 METHODOLOGY FOR ACTIVE INGREDIENT CONTENT (PIPERONYL BUTOXIDE)**

### **Outline of the method**

Piperonyl Butoxide content in the long lasting insecticide net sample was determined by GC-FID using CIPAC Volume – N, 33/LN/M/3.0 as reference method. The identity of the active ingredient was established by comparison with the equivalent authentic standard.

### **Preparation of Internal standard solution**

Weighed exactly 401.85 mg of octadecane, internal standard in a tare, glass weighing bottle and the same was transferred quantitatively in to 50 mL volumetric flask using Xylene. The solution was mixed well and was made up to the volume mark using Xylene. The solution was coded as “IS Solution”.

### **Preparation of calibration solution**

Weighed exactly 254.3 mg piperonyl butoxide reference standard in to a tare, glass weighing bottle and the same was transferred quantitatively in to a 50 mL volumetric flask using Xylene as solvent. The solution was coded as “Piperonyl Butoxide stock”. Accurately transferred using graduated pipette 0.25 mL, 1.50 mL, 2.00 mL, 3.00 mL, and 4.00 mL of “Piperonyl Butoxide stock” solution in to five separate 25 mL volumetric flasks. This was followed by addition of 2.0 mL of internal standard “IS solution” to each flask. The flasks were made up to the volume mark using Xylene and mixed well. The solutions were coded as CA, CB, CC, CD and CE respectively.

## 6.1 CHROMATOGRAPHIC SEPARATION PARAMETERS (PIPERONYL-BUTOXIDE)

Instrument	- Shimadzu Gas Chromatograph GC-2010 equipped with auto injector, Flame ionization detector and interfaced with GC solution software.
Detector	- FID
Column	- DB-1 Capillary Column (30m length x 0.32 mm I.D. x 0.25 $\mu$ film thickness).
Gas flow rate	
Column flow (N <sub>2</sub> )	- 1.50 ml/min
Hydrogen (H <sub>2</sub> )	- 40 ml/min
Air	- 400 ml/min
Make up (N <sub>2</sub> )	- 30 mL/min
Temperature	
Oven	- 180 °C hold 11 min, ramp @10°C/min 220°C hold 8 min, ramp @ -10°C/min 210°C hold 18 min, ramp@30°C/min 245°C hold 5 min
Injector	- 250 °C
Detector	- 300 °C
Volume injected	- 1 $\mu$ l
Injection Mode	- Split
Split ratio	- 25: 1
Retention time (approximate)	
Octadecane ( <i>Internal standard</i> )	- 6.2 min
Piperonyl Butoxide	- 23.0 min

## 6.2 DETERMINATION OF PIPERONYL BUTOXIDE (33/LN/M/3.0)

### Preparation of sample solution

The Duranet plus long lasting insecticide net incorporated with alpha-cypermethrin and piperonyl butoxide was weighed appropriately such a way that the quantization fits to linear range of calibration and quantitatively transferred to three separate 100 mL reflux flask. Each of the flask was added with 23 mL Xylene and 2 mL of “IS Solution”. The flasks were coded as S1, S2 and S3 with prefix label as Duranet plus. The flasks were connected to reflux condenser and refluxed for 30 min with stirring. The solutions in each flask were cooled to room temperature. The solution was filtered through 45 µm PTFE filter and a small aliquot was transferred to GC Vial.

### Linearity Determination

The calibration solutions CA, CB, CC, CD and CE were injected in to GC system equipped with FID. The detector response was recorded and a calibration curve was constructed by plotting the piperonyl butoxide to internal standard peak area ratio versus the mass of piperonyl butoxide in the calibration solutions.

### Sample Determination

The calibration solution was injected in followed by sample solutions in the sequence CB, S1, S2, S3, CB. The detector response was recorded and the piperonyl butoxide content in long lasting insecticide net (Duranet plus) was calculated.

$$\text{Piperonyl Butoxide content (g/kg)} = \frac{(R-b) \times P}{a \times w}$$

$$\text{Piperonyl Butoxide content (\%w/w)} = \frac{(R-b) \times P \times 100}{a \times w \times 1000}$$

Where,

CA, CB, CC, CD & CE	Calibration solutions
Hs	Peak area of PBO reference standard in calibration solution, ( $\mu\text{V}\cdot\text{Sec}$ )
Ir	Peak area of internal standard in calibration solution, ( $\mu\text{V}\cdot\text{Sec}$ )
Hw	Peak area of PBO in sample solution, ( $\mu\text{V}\cdot\text{Sec}$ )
Iq	Peak area of internal standard in sample solution, ( $\mu\text{V}\cdot\text{Sec}$ )
R	Peak area ratio Hs/Ir, Hw/Iq
w	Weight of net sample, (mg)
a	Slope obtained from linear regression equation
b	Intercept obtained from linear regression equation
P	Purity of PBO reference standard (g/kg)

## 7.0 WASH RESISTANCE INDEX CALCULATION (MT 195)

Calculate the wash resistance index, w, after the 4 washing-rinsing-heating cycles using the following equation:

$$w = 100 \times 4\sqrt{(t_4/t_0)}$$

where:

t<sub>4</sub> = total active ingredient content (in g/kg) after 4 washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces (Note 10)

t<sub>0</sub> = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces (Note 10)

w = wash resistance index, expressed as percentage (Note 11)

## 8.0 RESULTS

### **ALPHA-CYPERMETHRIN**

#### **Linearity of calibration solutions**

The detector response was found to be linear for the three calibration solutions injected in duplicates. The linear behaviour has been substantiated by the correlation co-efficient of 0.9999. The calibration details has been given in Table-1 and calibration curve in Figure-1.

#### **Quantification**

The calculations were carried out using the formula given in section 5.2. The results of calculations pertaining to before and after 4<sup>th</sup> wash samples were given in Table 3. Wash retention index calculated by section 6.3.

### **PIPERONYL BUTOXIDE**

#### **Linearity of calibration solutions**

The detector response was found to be linear for the five calibration solutions injected in GC-FID. The linear behaviour has been substantiated by the correlation co-efficient of 0.9999. The calibration details has been given in Table-2 and calibration curve in Figure-2.

#### **Quantification**

The calculations were carried out using the formula given in section 6.2. The results of calculations were given in Table 4. Wash retention index calculated by section 6.3.

## 9.0 CONCLUSION

The assay results of before wash and after 4<sup>th</sup> wash sample of alphacypermethrin (454/LN/M/3.2) and piperonyl butoxide (33/LN/M/3.0) of Duranet plus is as follows,

### ALPHA-CYPERMETHRIN

Sample Code	Concentration (g/kg)	
	Before wash	After 4 <sup>th</sup> wash
S1	6.05	5.78
S2	5.88	5.60
S3	5.64	5.87
<b>Average</b>	5.86	5.75
<b>Retention index</b>	99.54%	

### PIPERONYL BUTOXIDE

Sample Code	Concentration (g/kg)	
	Before wash	After 4 <sup>th</sup> wash
S1	2.32	1.78
S2	2.41	1.86
S3	2.38	1.82
<b>Average</b>	2.37	1.82
<b>Retention index</b>	93.56%	

**TABLE 1 LINEARITY MEASUREMENTS OF CALIBRATION SOLUTIONS  
(ALPHA-CYPERMETHRIN)**

Code	Concentration		Peak area ( $\mu\text{V}\cdot\text{Sec}$ )		Peak area
	(mg)	(mg/mL)*	Hs	Ir	ratio, Hs/Ir
CA	2.275	0.0226	9115	21417	0.4256
	2.275	0.0226	9512	22354	0.4255
CB	3.411	0.0339	14445	22587	0.6395
	3.411	0.0339	14366	22474	0.6392
CC	4.554	0.0452	19315	22635	0.8533
	4.554	0.0452	19563	23156	0.8448

\*Corrected for purity and calculated for unit volume

Slope - 0.1858

Intercept - 0.0037

Correlation Co-efficient - 0.9999

CA,CB,CC	Calibration solutions
Hs	Peak area of Alpha-cypermethrin reference standard in calibration solution, ( $\mu\text{V}\cdot\text{Sec}$ )
Ir	Peak area of internal standard in calibration solution, ( $\mu\text{V}\cdot\text{Sec}$ )
R	Peak area ratio, Hs/Ir

**TABLE 2** LINEARITY MEASUREMENTS OF CALIBRATION SOLUTIONS (PIPERONYL BUTOXIDE)

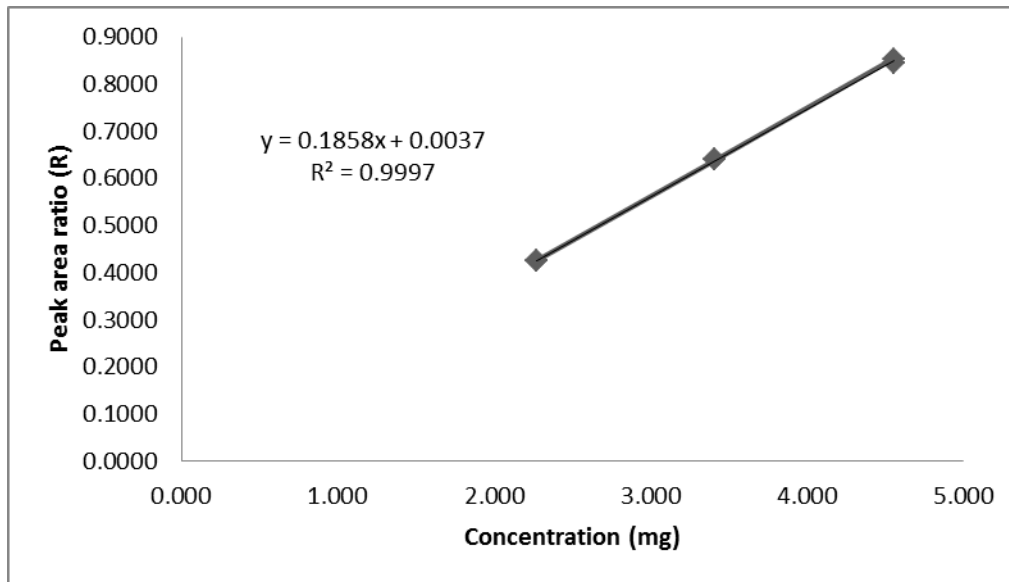
Code	Concentration		Peak Area ( $\mu\text{V} \cdot \text{Sec}$ )		Peak area ratio
	(mg)	(mg/mL)*	Hs	Ir	R
CA	1.27	0.0484	45126	185868	0.2428
CB	7.63	0.2909	308945	179585	1.7203
CC	10.17	0.3877	438867	185541	2.3653
CD	15.26	0.5817	645158	182568	3.5338
CE	20.34	0.7754	870055	182447	4.7688

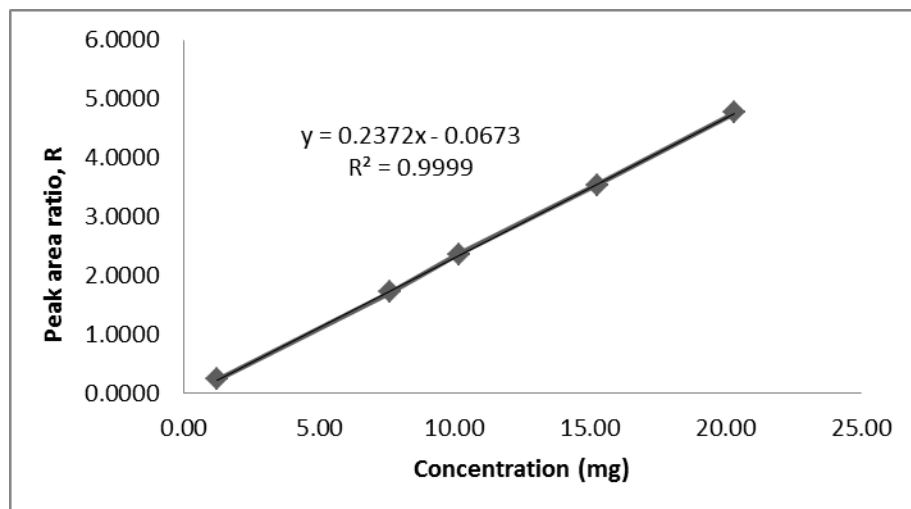
\*Corrected for purity and calculated for unit volume

Slope	-	0.2372
Intercept	-	-0.0673
Correlation Co-efficient	-	0.9999

CA,CB,CC,CD,CE	Calibration solutions
Hs	Peak area of Piperonyl butoxide reference standard in calibration solution, ( $\mu\text{V} \cdot \text{Sec}$ )
Ir	Peak area of internal standard in calibration solution, ( $\mu\text{V} \cdot \text{Sec}$ )
R	Peak area ratio, Hs/Ir



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

**FIGURE 2 CALIBRATION CURVE OF PIPERONYL BUTOXIDE**

**TABLE 3 CALCULATION DETAILS FOR ALPHA-CYPERMETHRIN CONTENT IN LONG LASTING INSECTICIDE NET (DURANET PLUS, BEFORE AND AFTER 4<sup>TH</sup> WASH)**

Code	Peak area ( $\mu\text{V}\cdot\text{Sec}$ )				s (mg)	P (g/kg)	w (mg)	f	Mean f	Content (g/kg)	Mean (g/kg)
	Hs	Ir	Hw	Iq							
<b>Quantification, Dura net Plus Before wash</b>											
CB	14984	23515			126.5	993		5322.56			
CB	14745	23325						5365.13			
S1			15247	23515			1149.2			6.05	
S2			13558	22845			1081.5		5359.31	5.88	5.86
S3			13369	23214			1095.2			5.64	
CB	15895	24945						5322.63			
CB	15154	24248						5426.90			
<b>Quantification, Dura net Plus After 4<sup>th</sup> wash</b>											
CB	17158	26745			126.5	993		5286.64			
CB	16287	26188						5453.37			
S1			16047	25247			1161.1			5.78	
S2			15845	25566			1168.4		5276.01	5.60	5.75
S3			15458	26587			1044.5			5.87	
CB	17845	26855						5104.02			
CB	16845	26125						5260.04			

NA = Not applicable

\*The value in parenthesis indicates alpha-cypermethrin content in percentage (w/w)

Statistics parameters	Before wash	After 4 <sup>th</sup> wash
Mean concentration (g/kg)	5.86	5.75
SD	0.21	0.14
%RSD	3.53	2.44
HL	2.90	2.91
Retention index (%)	99.54	

**TABLE 4 CALCULATION DETAILS FOR PIPERONYL BUTOXIDE CONTENT IN LONG LASTING INSECTICIDE NET (DURANET PLUS, BEFORE AND AFTER 4<sup>TH</sup> WASH)**

Code	Peak area ( $\mu\text{V}\cdot\text{Sec}$ )				peak area	Purity	Sample	Slope	Intercept	Content	
	Hs	Ir	Hw	Iq	ratio, R	(g/kg)	Wt.(w,mg)	a	b	(g/kg)	Mean (g/kg)
<b>Quantification, Dura net Plus Before wash</b>											
CB	314675	183458			1.7152						
S1			90514	180154	0.5024	953	985.5	0.2372	-0.0673	2.3228	2.37
S2			91447	179474	0.5095	953	960.5	0.2372	-0.0673	2.4130	
S3			90256	178565	0.5055	953	965.5	0.2372	-0.0673	2.3835	
CB	318747	181447			1.7567						
<b>Quantification, Dura net Plus After 4<sup>th</sup> wash</b>											
CB	319856	185658			1.7228						
S1			68587	185447	0.3698	953	985.8	0.2372	-0.0673	1.7817	1.82
S2			71214	184747	0.3855	953	979.6	0.2372	-0.0673	1.8571	
S3			67847	183474	0.3698	953	966.6	0.2372	-0.0673	1.8169	
CB	322145	187457			1.7185						

NA = Not applicable

\*The value in parenthesis indicates alpha-cypermethrin content in percentage (w/w)

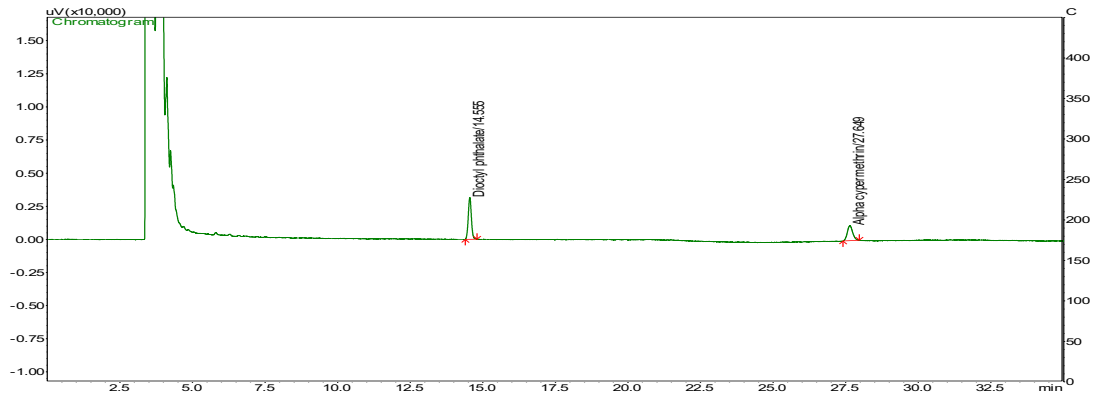
Statistics parameters	Before wash	After 4 <sup>th</sup> wash
Mean concentration (g/kg)	2.37	1.82
SD	0.05	0.04
%RSD	1.94	2.07
HL	3.33	3.46
Retention index (%)	93.56	

# APPENDIX – I

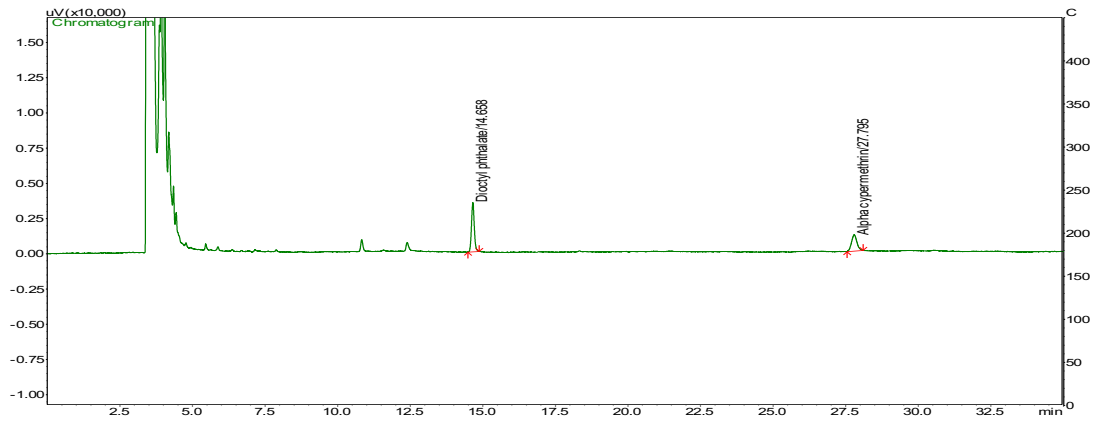
# CHROMATOGRAMS

## ALPHA-CYPERMETHRIN

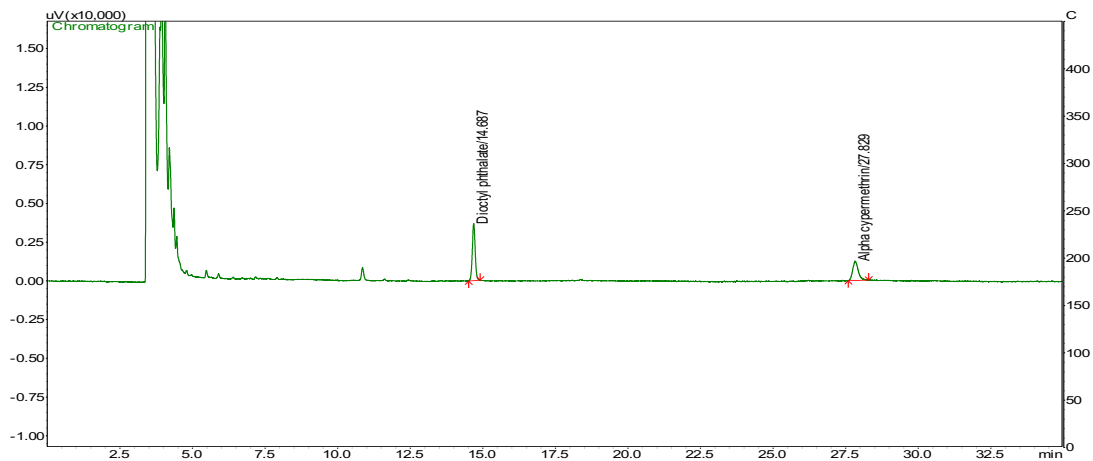
### Alpha-cypermethrin Standard [CB]



### DuraNet Sample (Before Wash)

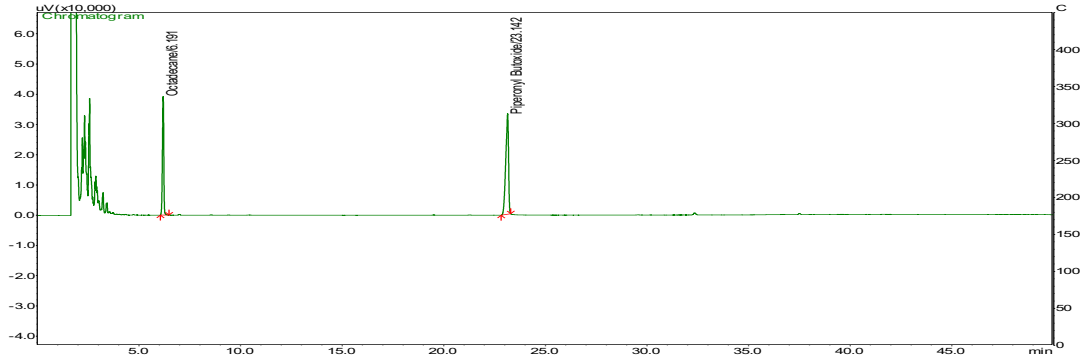


### DuraNet Sample (After 4th wash)

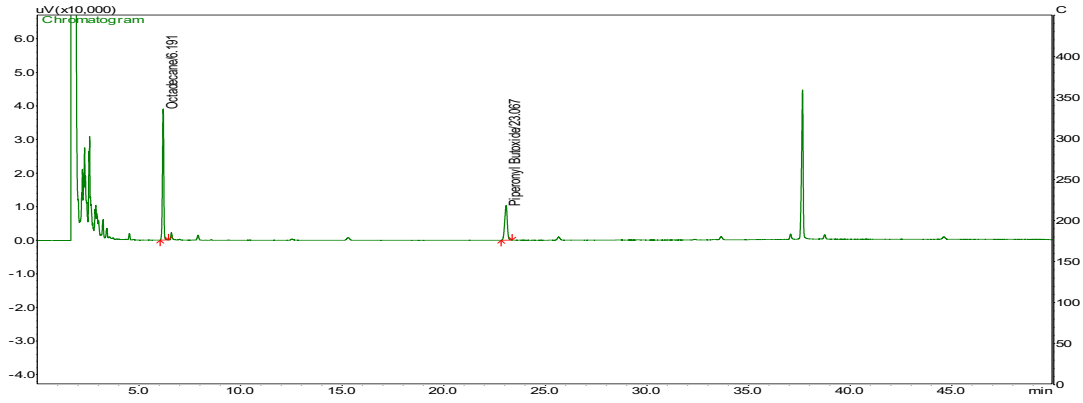


# Piperonyl butoxide

## Piperonyl Butoxide Standard [CB]



## DuraNet Sample (Before Wash)



## DuraNet Sample (After 4th wash)

