DISEASE CONTROL TECHNOLOGIES

DETERMINATION OF PYRIPROXYFEN AND ALPHA-CYPERMETHRIN CONTENT INCORPORATED IN ROYAL GUARD™ HDPE LONG LASTING INSECTICIDE TREATED NETS (LLINs)

1· Scope of study

The study is in fulfillment of method validation and quantification requirements for the evaluation of Pyriproxyfen and Alpha-Cypermethrin in Royal Guard™ long lasting insecticidal nets. The study was conducted by IIBAT in collaboration with M/s Clariant International Limited on behalf of Disease Control Technologies LLC.

2. Test item of the study

Royal Guard™ long lasting insecticidal nets, incorporating both Pryriproxyfen and Alpha-Cypermethrin active ingredients were supplied by Disease Control Technologies LLC with the following general specifications.

1. Royal Guard™ – 120 Denier (38 GSM fabric, 5.5 g/kg PPF and ALPHA)
2. Royal Guard™ – 150 Denier (45 GSM fabric, 5.0 g/kg PPF and ALPHA)

3. Validation of the analytical method

Validation of the analytical method for determination of Pyriproxyfen and Alpha-Cypermethrin was performed during the month of May 2016.

3.1 Specificity

The instrument response of solvents (n-Heptane, 2-propanol, acetonitrile and milliQ water) was compared with response of reference analytical standards (pyriproxyfen, alpha-cypermethrin and dicyclohexyl phthalate) and extract of long lasting mosquito net. The results reveal either the solvents or the impurities of the long lasting insecticidal net interfered with the response of analyte of interest at specified retention time.

3.2 Linearity of the detector response

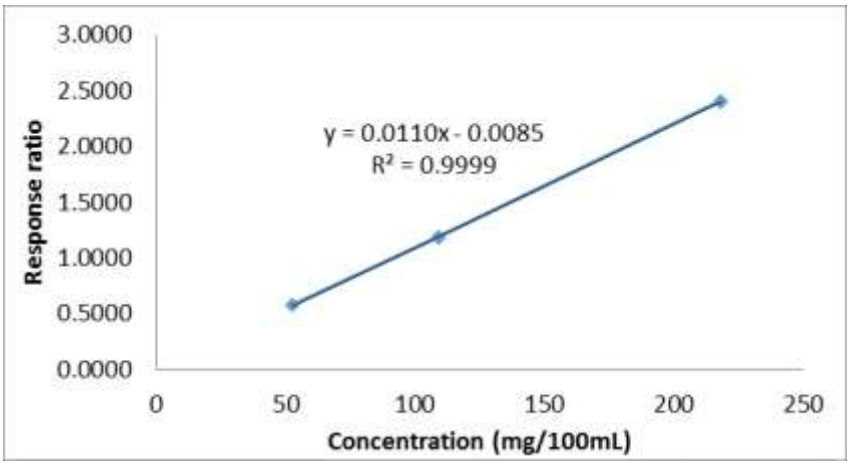
The instrument response was found to be linear against the concentration of pyriproxyfen and alpha-cypermethrin. The linearity was demonstrated over a range of concentrations from 52mg/100ml to 218mg/100ml and 52mg/100 mL to 220 mg/100mL for pyriproxyfen and alpha-cypermethrin respectively. Three linear concentration solutions representing approximately 0.5, 1.0 and 2.0 times the concentration of pyriproxyfen and alpha-cypermethrin in the long lasting insecticidal net were prepared for linearity determination. Three replicate injections for each concentration were carried out to evaluate the linear behavior of instrument response against concentration of analyte. The instrument response factor (response ratio of pyriproxyfen Vs dicyclohexylpthalate and response ratio of alpha-cypermethrin Vs dicyclohexylpthalate) was correlated against concentration of pyriproxyfen and alpha-cypermethrin reference standard solutions.

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 0.9999 and 1.0000 for pyriproxyfen and alpha-cypermethrin respectively. The results are summarized as follows.

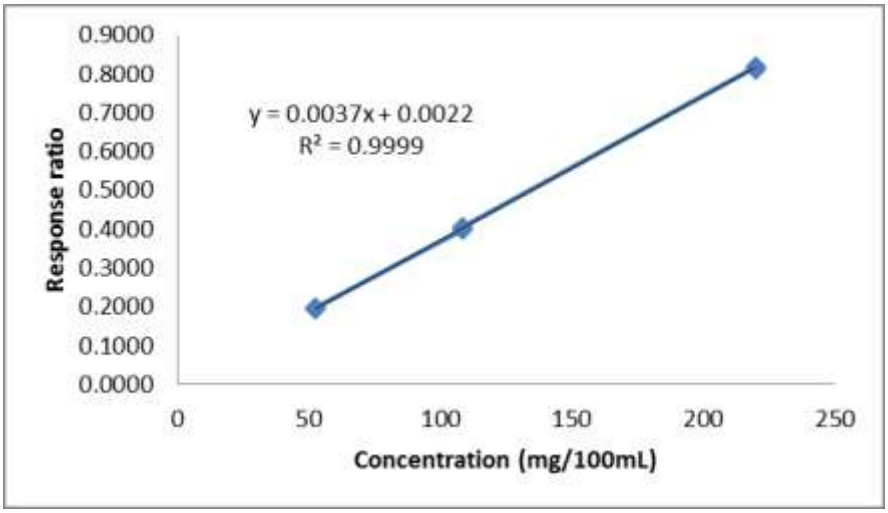
LINEARITY DATA

Pyriproxyfen			Alpha-Cypermethrin		
Code	**Concentration (mg/100 mL)**	**Response Ratio**	**Code**	**Concentration (mg/100 mL)**	**Response Ratio**
CA	52.56	0.5776	CA	52.66	0.1972
CB	109.25	1.1848	CB	108.32	0.4028
CC	218.35	2.4012	CC	219.81	0.8157
Correlation Co-efficient		0.9999	Correlation Co-efficient		1.0000
Slope		0.0110	Slope		0.0037
Intercept		-0.0085	Intercept		0.0021

PYRIPROXYFEN



ALPHA-CYPERMETHRIN



3.3 Repeatability

The repeatability of the method was assessed by performing 6 replicate determinations of pyriproxyfen and alpha-cypermethrin in long lasting insecticidal net. The results are summarized as follows.

Royal Guard™ 120D, 38 GSM Fabric			
Code	**Pyriproxyfen (g/kg)**	**Code**	**Alpha-Cypermethrin (g/kg)**
P1	5.40	P1	5.31
P2	5.37	P2	5.28
P3	5.37	P3	5.27
P4	5.38	P4	5.35
P5	5.42	P5	5.31
P6	5.32	P6	5.36
Mean	**5.38**	**Mean**	**5.31**
SD	**0.03**	**SD**	**0.04**
%RSD	**0.58**	**%RSD**	**0.71**
HL	**2.94**	**HL**	**2.95**

Royal Guard™ 150D, 38 GSM Fabric			
Code	**Pyriproxyfen (g/kg)**	**Code**	**Alpha-Cypermethrin (g/kg)**
P1	4.90	P1	4.89
P2	4.86	P2	4.93
P3	4.85	P3	4.82
P4	4.83	P4	4.80
P5	4.83	P5	4.85
P6	4.80	P6	4.80
Mean	**4.85**	**Mean**	**4.85**
SD	**0.03**	**SD**	**0.05**
%RSD	**0.68**	**%RSD**	**1.08**
HL	**2.99**	**HL**	**2.99**

3.4 Accuracy

Assay accuracy of method determination was calculated by fortification of appropriate concentration equal to the concentration available in long lasting insecticidal net. The recovery results are summarized as follows.

Royal Guard™ 120D, 38 GSM Fabric: Recovery Details			
Code	**Pyriproxyfen Recovered (%)**	**Code**	**Alpha-Cypermethrin Recovered (%)**
T1R1	98.95	T1R1	96.80
T1R2	97.65	T1R2	98.82
T1R3	97.02	T1R3	97.80
T1R4	98.59	T1R4	98.62
Mean	**98.05**	**Mean**	**98.01**
SD	**0.88**	**SD**	**0.92**
%RSD	**0.90**	**%RSD**	**0.94**
HL	**1.34**	**HL**	**1.34**

Royal Guard™ 150D, 38 GSM Fabric: Recovery Details			
Code	**Pyriproxyfen Recovered (%)**	**Code**	**Alpha-Cypermethrin Recovered (%)**
T2R1	99.37	T2R1	96.96
T2R2	98.34	T2R2	97.59
T2R3	97.36	T2R3	98.67
T2R4	96.65	T2R4	98.29
Mean	**97.93**	**Mean**	**97.88**
SD	**1.18**	**SD**	**0.76**
%RSD	**1.21**	**%RSD**	**0.77**
HL	**1.34**	**HL**	**1.34**

4.0 Quantification

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.

Royal Guard™ 120D, 38 GSM Fabric			
Code	**Pyriproxyfen (g/kg)**	**Code**	**Alpha-Cypermethrin (g/kg)**
SA1	5.32	SA1	5.33
SA2	5.27	SA2	5.42
SB1	5.44	SB1	5.40
SB2	5.44	SB2	5.31
Mean	**5.37**	**Mean**	**5.36**
SD	**0.09**	**SD**	**0.05**
%RSD	**1.61**	**%RSD**	**0.98**
HL	**2.94**	**HL**	**2.94**

Royal Guard 150D, 38 GSM Fabric			
Code	**Pyriproxyfen (g/kg)**	**Code**	**Alpha-Cypermethrin (g/kg)**
SA1	4.87	SA1	4.73
SA2	4.84	SA2	4.73
SB1	4.89	SB1	4.80
SB2	4.91	SB2	4.84
Mean	**4.88**	**Mean**	**4.77**
SD	**0.03**	**SD**	**1.06**
%RSD	**0.68**	**%RSD**	**1.17**
HL	**2.99**	**HL**	**3.00**

Note:
The values provided in this report are calculated using excel spread sheet and truncated to two decimals without rounding off.

Abbreviation

SD	=	Standard deviation
RSD	=	Relative standard deviation
HL	=	Modified Horwitz limit

CHROMATOGRAMS OF CONTROL (NET SAMPLE)

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Study No          :16020
Instrument ID     :AC/HPLC/93
Data file name    :D:\PYRIPROXYFEN\SPECIFICITY NET\PYRI 004.D
Method File Name  :D:\PYRIPROXYFEN\PYRIPROXYFEN_LC.M
Compound Name     :Pyriproxyfen                                    ->
Sample Name       :Control net sample
Injection Date    :5/4/2016
Injection Time    :9:05:36 PM

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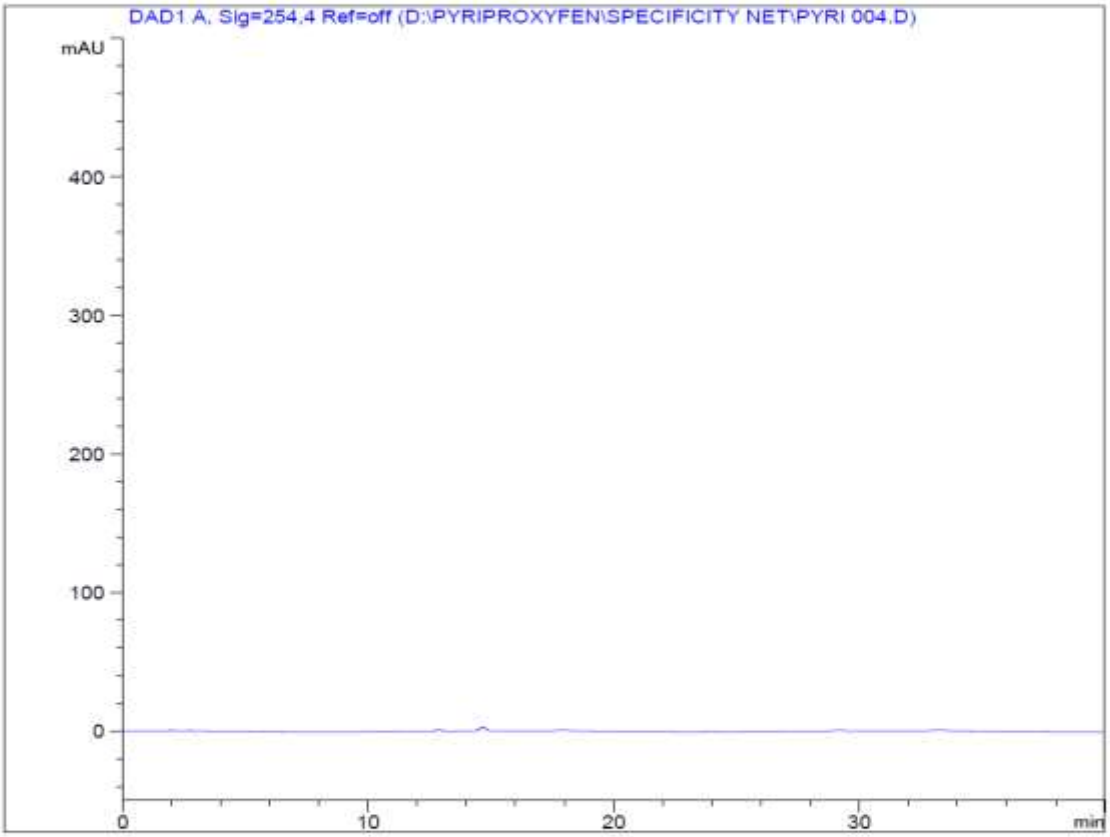

Customized Report

Signal 1 :DAD1 A, Sig=254,4 Ref=off

Peak	Compound Name	RT[min]	Area
Totals:			

*** End of the Report ***

CHROMATOGRAM OF CALIBRATION STANDARD (CB)

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Study No            :16020
Instrument ID       :AC/HPLC/93
Data file name      :D:\PYRIPROXYFEN\LINEARITY\PYRI 0212.D
Method File Name    :D:\PYRIPROXYFEN\PYRIPROXYFEN_LC.M
Compound Name       :Pyriproxyfen                                          ->
Sample Name         :CB
Injection Date      :5/5/2016
Injection Time      :4:39:08 AM

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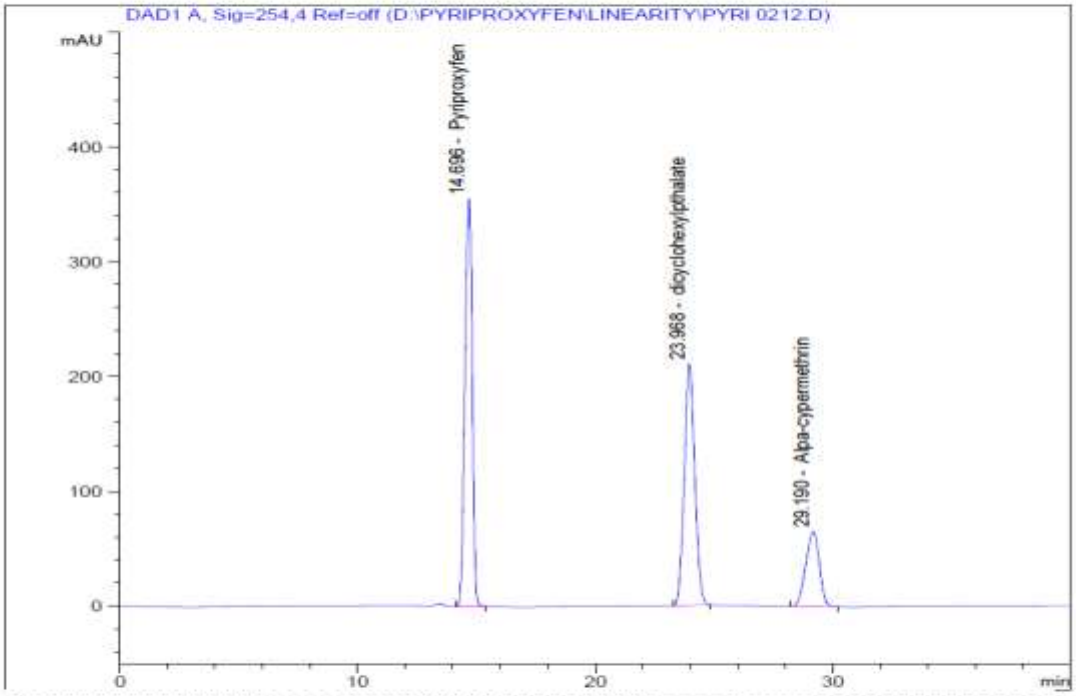

Customized Report

Signal 1 :DAD1 A, Sig=254,4 Ref=off

Peak	Compound Name	RT[min]	Area
1	Pyriproxyfen	14.696	7544.06
2	dicyclohexylpthalate	23.968	6396.68
3	Alpa-cypermethrin	29.190	2575.15

Totals:

*** End of the Report ***

CHROMATOGRAM OF ROYAL GUARD™ 120D LLIN

```
Study No            :16020
Instrument ID       :AC/HPLC/93
Data file name      :D:\PYRIPROXYFEN\QUANTIFICATION120D\PYRI 002.D
Method File Name    :D:\PYRIPROXYFEN\PYRIPROXYFEN_LC.M
Compound Name       :Pyriproxyfen                                        ->
Sample Name         :120-D/SA1
Injection Date      :5/7/2016
Injection Time      :11:18:03 AM

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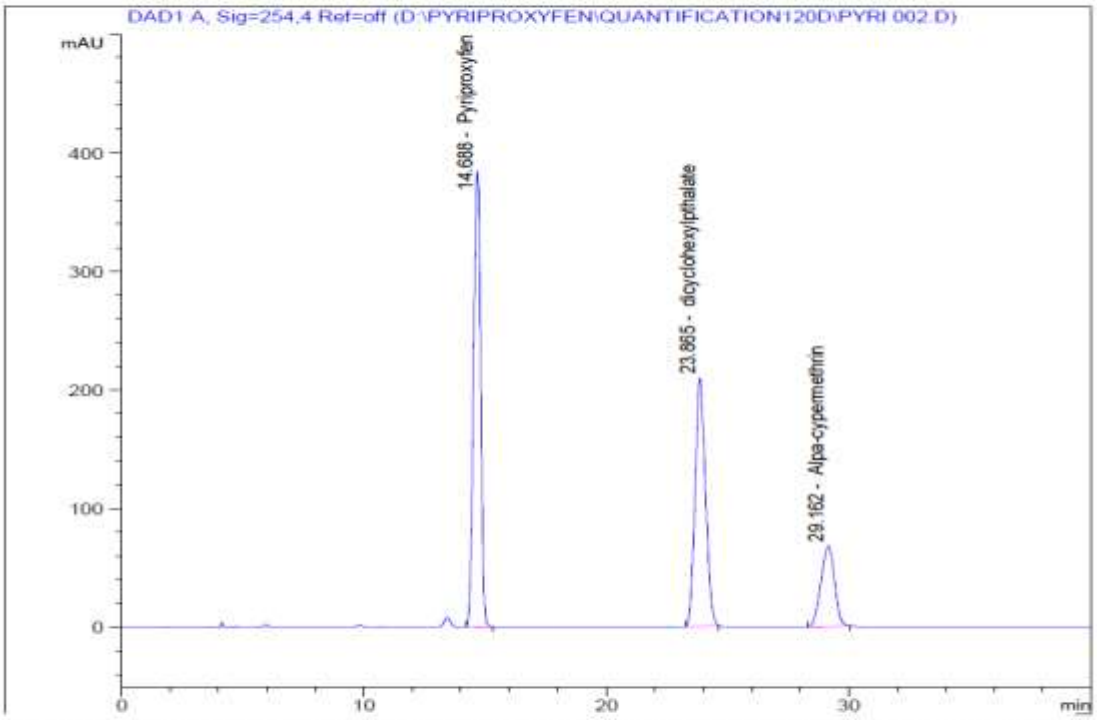

Customized Report

Signal 1 :DAD1 A, Sig=254,4 Ref=off

Peak	Compound Name	RT[min]	Area
1	Pyriproxyfen	14.688	7830.33
2	dicyclohexylpthalate	23.865	6177.80
3	Alpa-cypermethrin	29.162	2639.37

Totals:

*** End of the Report ***

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CHROMATOGRAM OF ROYAL GUARD™ 150D LLIN

```
Study No          :16020
Instrument ID     :AC/HPLC/93
Data file name    :D:\PYRIPROXYFEN\QUANTIFICATION150D\PYRI 002.D
Method File Name  :D:\PYRIPROXYFEN\PYRIPROXYFEN_LC.M
Compound Name     :Pyriproxyfen                                    ->
Sample Name       :150-D/SA1
Injection Date    :5/8/2016
Injection Time    :1:28:22 AM

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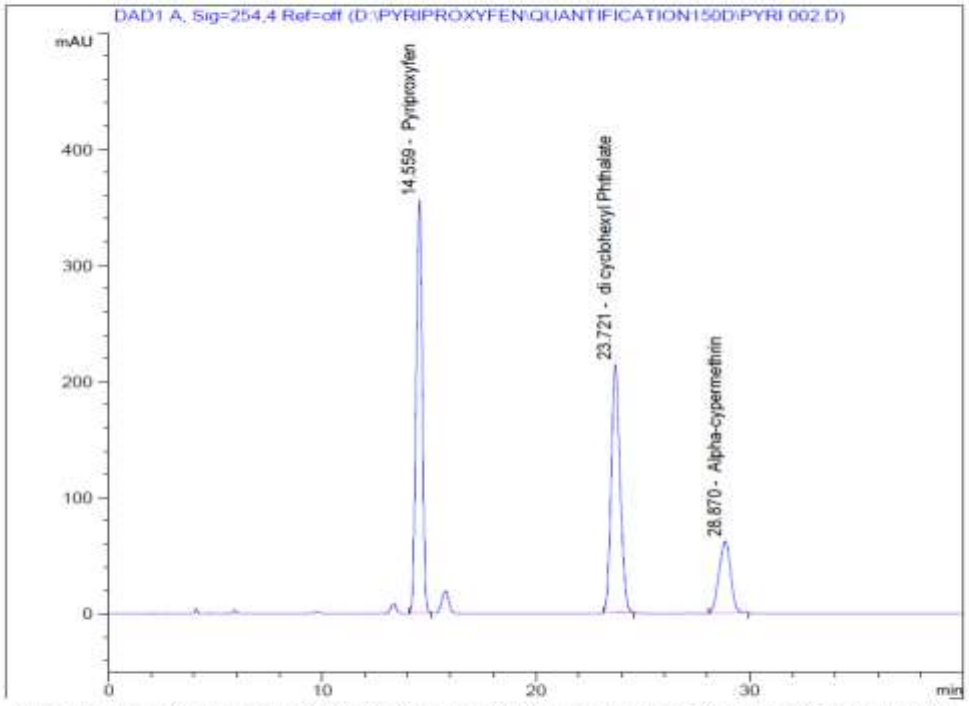

Customized Report

Signal 1 :DAD1 A, Sig=254,4 Ref=off

Peak	Compound Name	RT[min]	Area
1	Pyriproxyfen	14.559	7062.87
2	di cyclohexyl Phthalate	23.721	6141.25
3	Alpha-cypermethrin	28.870	2326.59

Totals:

*** End of the Report ***