METHOD EXTENSION FOR THE DETERMINATION OF PYRIPROXYFEN AND ALPHA-CYPERMETHRIN IN LONG LASTING INSECTICIDAL NET

INTRODUCTION

The content of pyriproxyfen and alpha-cypermethrin in long lasting net has been determined with reference to the method CIPAC/4887/R validated for pyriproxyfen and permethrin. The method of determination is an extension of the CIPAC/4887/R. The method has been developed covering the validation parameters such as specificity, linearity of response, repeatability of determination, and assay accuracy.

METHOD DESCRIPTION

Outline of Method

The pyriproxyfen and alpha-cypermethrin in long lasting insecticidal net is determined by reverse phase high performance liquid chromatography using PDA detector, at detection wavelength of 254 nm with dicyclohexyl phthalate as internal standard.

Reagents

Heptane

2-propanol

Acetonitrile HPLC Grade

Water HPLC grade

Pyriproxyfen and alpha-cypermethrin reference analytical standard of known purity, stored in refrigerator.

Apparatus

- High performance liquid chromatography (HPLC) equipped with detector suitable for operation at 254 nm, a constant temperature column compartment and an injector capable of delivering 10µL.
- HPLC column of specification 250 mm x 4.6 mm, C18 (5 µm) or equivalent
- Water bath
- Rotary evaporator

PROCEDURE

Preparation of Internal Standard

Dicyclohexyl phthalate has been used as an internal standard. 5.0 g of dicyclohexyl phthalate is weighed and transferred in to 200 mL volumetric flask. It is dissolved using 2-propanol and diluted to the volume using the same solvent.

Preparation of Calibration Solutions

It is weighed in duplicate 90 to 110 mg of pyriproxyfen and alpha-cypermethrin reference analytical standard in to separate 200 mL volumetric flasks. Added 10 mL internal standard solution by pipette to each vial or flask, and by measuring cylinder acetonitrile, 90mL. Mixed well. Prepared calibration solutions 0.5, 1 and 2 times that of calibration solution.

Preparation of Sample Solutions

The LLIN has been cut in to small pieces of 5-10 mm squares using acetone washed and rinsed scissors. The test item has been weighed sufficient enough to contain 18-22 mg (w, mg) of pyriproxyfen and alpha-cypermethrin in to a vial / stoppered flask (100 mL). The internal standard solution 2.0 mL has been added followed by 48 mL of n-Heptane. The vial/stoppered flask has been shifted to water bath equilibrated at 87°C±3°C with a condenser (circulated with liquid maintained at 10°C) fitted to flask. The digestion has been continued for 1 hour with occasional shaking three times during the extraction process. After extraction the solution has been cooled to room temperature without removing the condenser. After attaining room temperature, 25 mL of the solution has been transferred to ground joint round – bottomed flask followed by concentration to near dryness using rotary evaporator under vacuum. Diluted using 10 mL of acetonitrile, filter and inject the sample solutions to get the response (solutions SA and SB).

Instrumentation Conditions

Column	:	250 mm x 4.6 mm (id) x 5µm, C18 column or equivalent
Mobile phase	:	Acetonitrile (67%) and water (33%)
Column temperature	:	40°C
Flow rate	:	1.0 mL/min
Detector wavelength	:	254 nm
Injection volume	:	10 µL

Retention times (Approximately):

Pyriproxyfen	:	17 minutes
Dicyclohexyl phthalate	:	25 minutes
Alpha-Cypermethrin	:	30 minutes

VALIDATION PARAMETERS

Specificity

The solvents, LLIN extract solution, reference standards and internal standard solutions separately been compared with their individual response to any interference with the response of analyte *viz,* pyriproxyfen, alpha-cypermethrin and dicyclohexyl phthalate.

Method precision

Six separate sub-samples from the sample of pyriproxyfen and alpha-cypermethrin LLIN have been analyzed following the extraction procedure and the conditions mentioned. The repeatability of this method has been evaluated statistically.

Accuracy

The stock solution at appropriate concentrations of pyriproxyfen and alpha-cypermethrin has been fortified to the blank formulation so that the fortified concentrations of Pyriproxyfen and alpha-cypermethrin were at the levels of each specification. The recovery of both active analyte have been calculated and evaluated statistically.

Quantification

The quantification has been carried out using analytical reference standard as bracketing solution in the following sequence Calibration Solution CA, Sample Solution SA, Sample Solution SA, Calibration Colution CB, Sample Solution SB, Sample Solution SB, Calibration Solution CA.

The mean value of the response factors of calibration solution bracketing two sample solutions have been used to calculate the Pyriproxyfen and alpha-cypermethrin concentration.

CALCULATION PYRIPROXYFEN AND ALPHA-CYPERMETHRIN

Pyriproxyfen

fi	=	(Ir x s x P) / (Hs x 5)
Pyriproxyfen concentration	=	(f x Hw) / (Iq x w) g/kg

Alpha-cypermethrin

fi	=	(Ir x s x P) / (Hs x 5)
Alpha-cypermethrin concentration	=	(f x Hw) / (Iq x w) g/kg

Where,

fi	=	Individual response factor
f	=	Mean response factor
Hs	=	Peak area of pyriproxyfen / alpha-cypermethrin in Calibration solution
Hw	=	Peak area of pyriproxyfen / alpha-cypermethrin 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 pyriproxyfen / alpha-cypermethrin in sample Solution (mg)
w	=	Mass of sample taken (mg)
P	=	Purity of pyriproxyfen / alpha-cypermethrin (g/kg)

CONCLUSION

We propose the CIPAC/ 4887/R extension method for LLIN, when active substances, pyriproxyfen and alpha-cypermethrin are incorporated in HDPE polymer, as there is only minor difference in retention time in the method of high performance liquid chromatography (HPLC) using PDA detector.

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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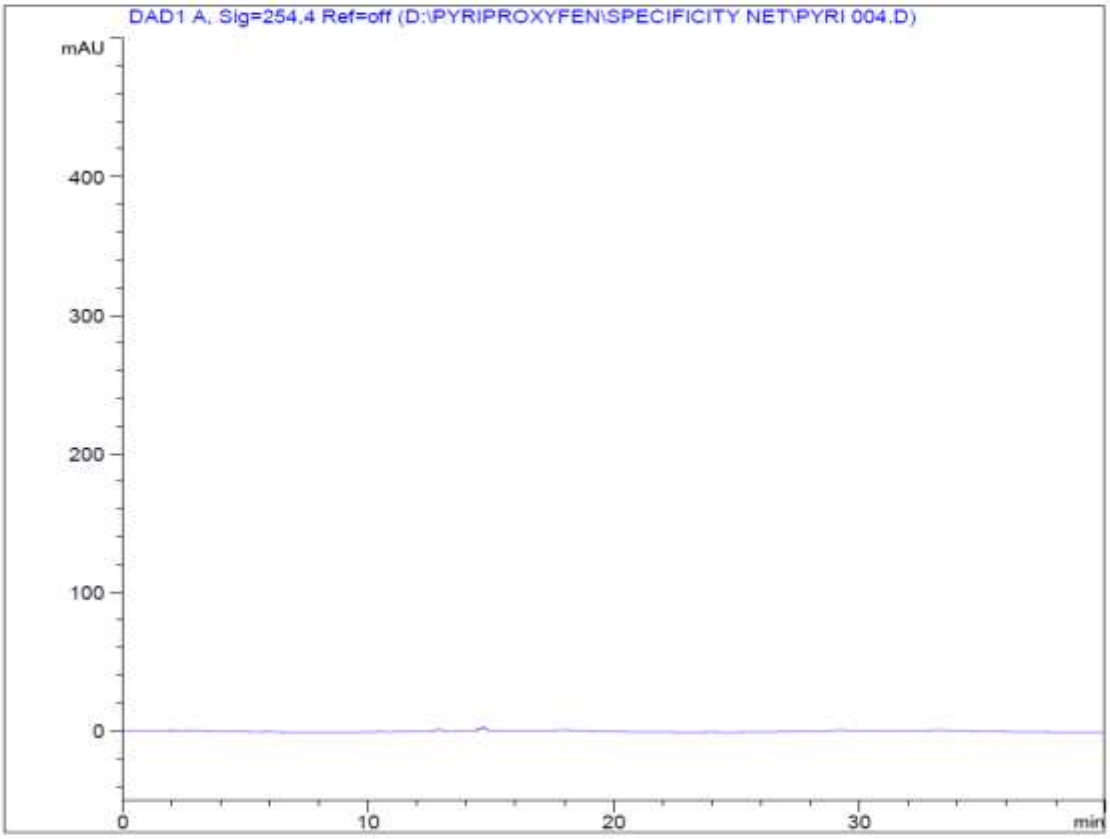

Customized Report

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)

```
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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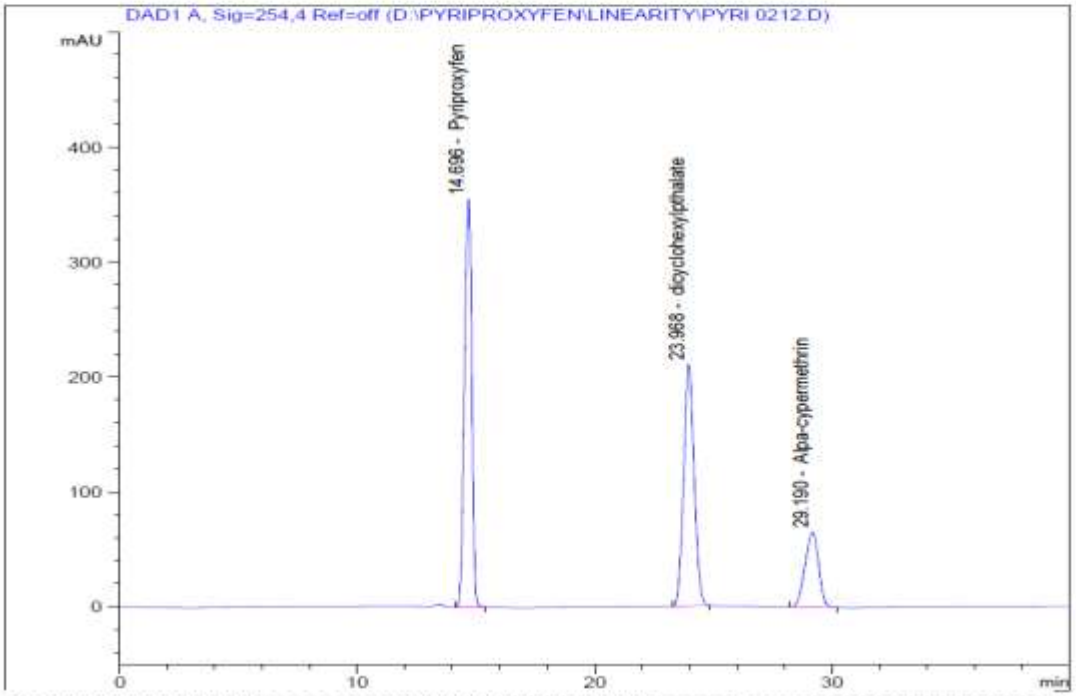

Customized Report

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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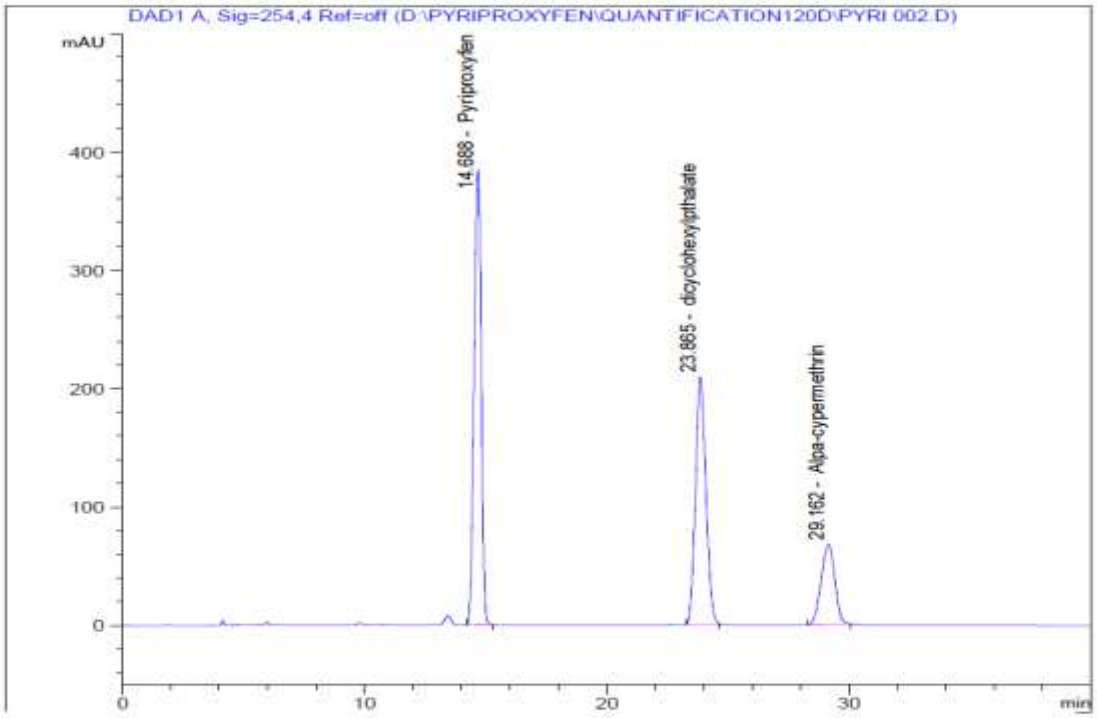

Customized Report

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 ***

CHROMATOGRAM OF ROYAL GUARD™ 150D LLIN

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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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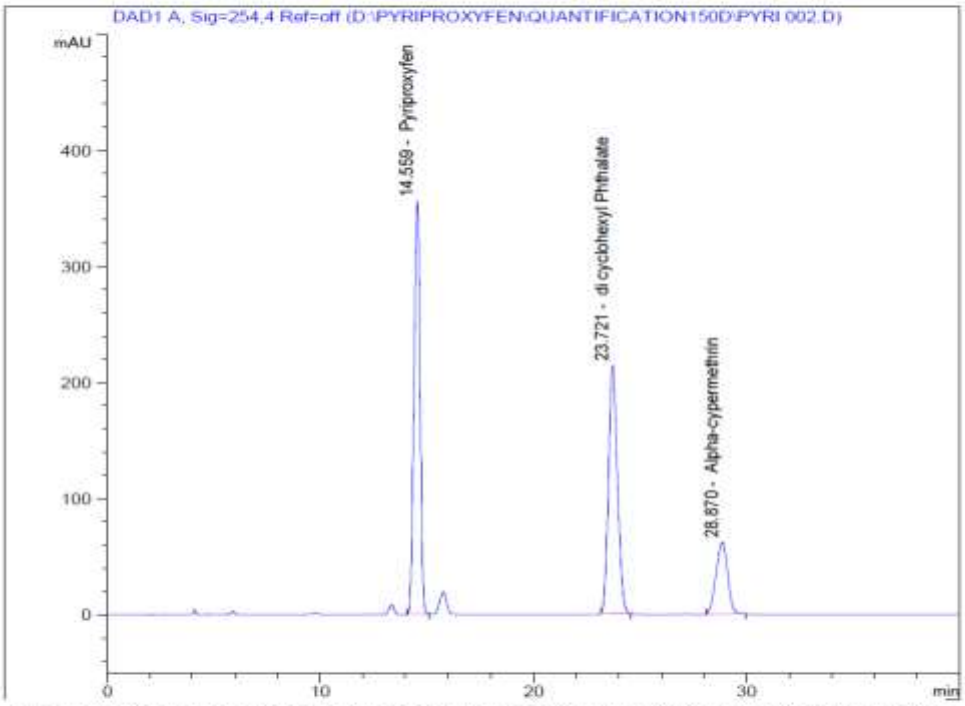

Customized Report

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 ***