CHLORFENAPYR + ALPHACYPERMETHRIN

EXTENSION OF SCOPOE OF THE METHOD CIPAC 5220/m

TO INSECTICIDE INCORPORATED MOSQUITO NETS

CIPAC method extension study for the determination of

CHLORFENAPYR + ALPHACYPERMETHRIN

IN LONG LASTING INSECTICIDAL NET BY GC

Report to CIPAC by

SAMBHV LIFE SCIENCE PRIVATE LIMITED

5th Floor, 501, Block C, Harshit Corporate, Aamnaka,

Raipur, Chhattisgarh-India

1. General information:

- **ALPHA-CYPERMETHRIN**

Common name : alpha-cypermethrin (E-ISO, BSI), alpha-cyperméthrine (F-ISO)

Synonyms : alphamethrin (rejected common name), alfoxylate

Chemical names

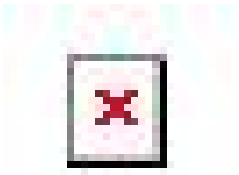
IUPAC a racemic mixture of: (S)-α-cyano-3-phenoxybenzyl-(1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl-(1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CA: [1α(S*),3α]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Empirical formula

$C_{22}H_{19}Cl_2NO_3$

Structural formula :



Relative molecular mass : 416.3

CAS Registry number

67375-30-8

CIPAC number

454

Identity tests

GC retention time, IR spectrum

-Chlorfenapyr

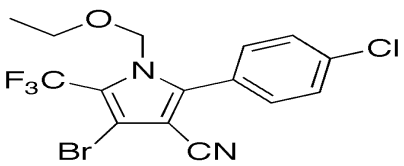
ISO common name : chlorfenapyr (BSI, E-ISO, ANSI); chlorfénapyr ((m) F-ISO)

Chemical names

IUPAC 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1*H*-pyrrole-3-carbonitrile

CA 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1*H*-pyrrole-3-carbonitrile

Structural formula



Empirical formula : C15H11BrClF3N2O

Relative molecular mass

407.6

CAS Registry number

122453-73-0

CIPAC number

570

Identity tests

HPLC retention time, IR spectrum

2. Details of samples

Batch 1

Batch 2

Batch 3

Batch 4

Batch 5

3. Scope: Extension of Scope of method CIPAC 5220/m to incorporated ITN

4. Details of analytical method: CIPAC 5220/m

Handbook P, p.62 to Chlorfenapyr 6.0 g/Kg + Alphacypermethrin 4.0 g/Kg

5. Details of Minor changes:

CIPAC Book P, Page 62 , No 5221	**Minor changes**
2. Preparation of sample (LN)	**2.Preparation of Sample (LN)**
Weighed in duplicate, accurately to the nearest 0.1 mg, a sufficient amount of sample containing about 1.2 to 3.4 mg of Alpha-cypermethrin and about 2.4 to 3.2 mg of Chlorfenapyr (corresponding to about 500 mg of sample of a treated net such as Interceptor® G2, Mont Inari Dual 1 and Mont Inari Dual 2) into separate 100 mL glass bottles. Added precisely 1 mL of the internal standard solution CISTD and about 24 mL of heptane. Placed the flasks in an ultrasonic bath for 30 minutes. Allowed the flasks to cool to room temperature and filtered an aliquot of each solution through a 0.45 µm pore size filter into a GC injection vial*. Added about 50 µL of the citric acid solution to avoid epimerization of Alpha-cypermethrin during the analysis. Sealed and shaken the vials.	An aliquot of sample containing about 1.2 to 3.4 mg alpha-cypermethrin and about 2.4 to 3.2 mg chlorfenapyr will be weighed in duplicate, (accurately to the nearest 0.1 mg), into separate 50 or 100 mL glass bottles (e.g. "Schott®" type) or disposable flasks (e.g. 50 mL conical tubes). To this 1 mL of the internal standard solution and 24 mL of heptane will be added. The flasks will be placed in an ultrasonic bath at 90°C for 30 minutes. The flasks will be allowed to cool to room temperature and filter an aliquot of each solution through a 0.45 µm pore size filter into a GC injection vial. Add about 50 µL of the citric acid solution to avoid epimerization of alpha-cypermethrin during analysis

6. **Procedure for the collaborative trial**

The samples were analyzed on two different days, each day involving duplicate injections of duplicate weights. Both test and reference solutions were freshly prepared on each day.

7. Analytical Conditions

Instrument "Shimadzu Gas Chromatograph Model 2010 equipped with Auto injector AOC 20i, connected with GC solutions software"

Detector Flame Ionization Detector (FID)

Column DB-1 Capillary Column (30 m length x 0.25 mm I.D. x 0.25 µm film thickness).

Injection System

Injector Split injection

Injector Temperature 260°C

Split ratio 10:01

Injection volume 1 µL

Detector Temperature 300 °C

Temperature Column oven 180°C for 0.5 minutes, Ramp 20°C/min to 280°C, Hold for 5 minutes

Nitrogen (Carrier gas) 0.85 mL/min

Air flow 400 mL/min

Hydrogen flow 30 mL/min

Nitrogen (Make up) 30 mL/min

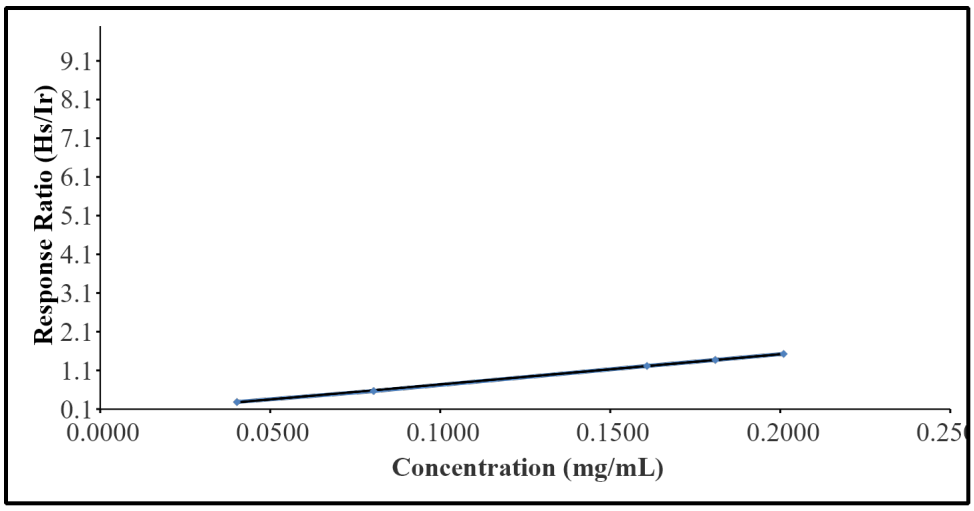
Retention time (approximate)

Chlorfenapyr 5.8 minutes

Dicyclohexyl phthalate (Internal standard) 7.3 minutes

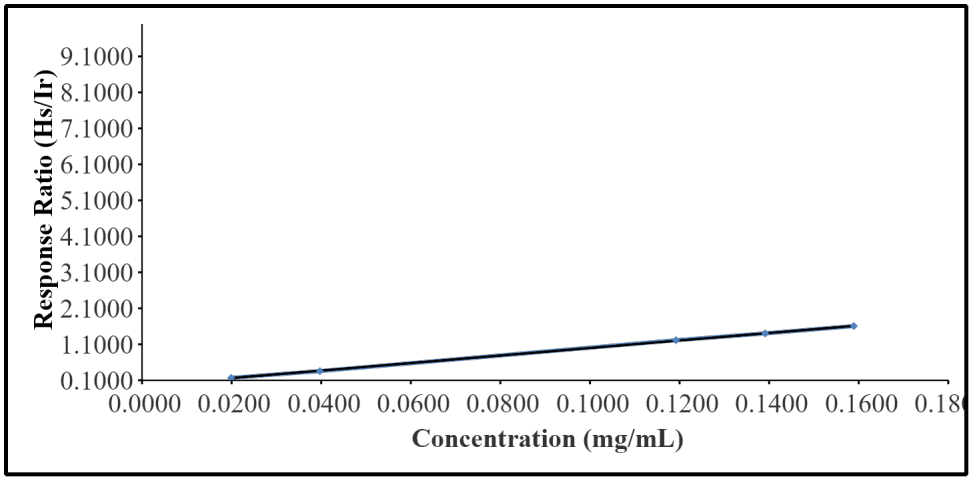
Alpha-cypermethrin 9.8 minutes

8. Linearity



Linearity Curve for Chlorfenapyr					
Sample	**Weight s***	**Concentration**	**Hs**	**Ir**	**Response Ratio**

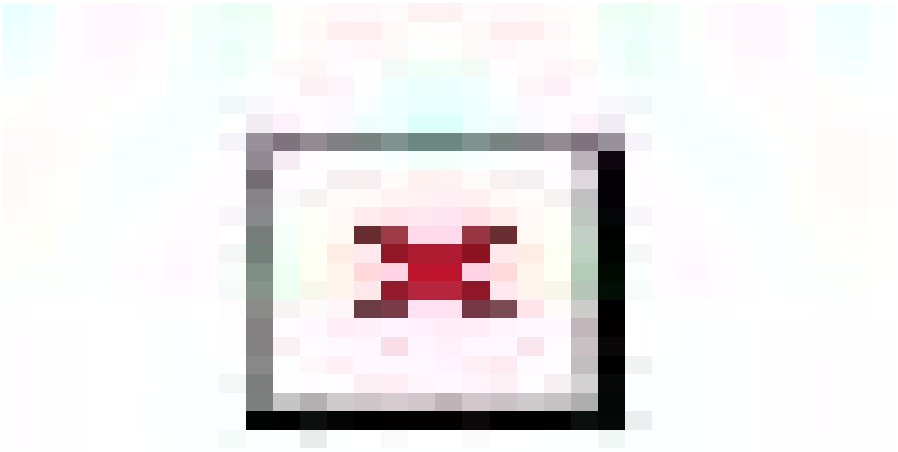
Code	(mg)	(mg/mL)	(µV*Sec)	(µV*Sec)	(Hs/Ir)
C1	1.0049	0.0402	38625	136254	0.2835
C2	2.0099	0.0804	83386	147142	0.5667
C3	4.0197	0.1608	174625	143769	1.2146
C4	4.5222	0.1809	194487	141852	1.3711
C5	5.0247	0.2010	217698	142524	1.5274
Slope					**7.8071**
Intercept					**-0.0430**
CC					**0.9998**



Linearity Curve for Alpha-cypermethrin					
Sample	**Weight s***	**Concentration**	**Hs**	**Ir**	**Response Ratio**
Code	**(mg)**	**(mg/mL)**	**(µV*Sec)**	**(µV*Sec)**	**(Hs/Ir)**
C1	0.4967	0.0199	22785	136254	0.1672
C2	0.9933	0.0397	52259	147142	0.3552
C3	2.9800	0.1192	174215	143769	1.2118
C4	3.4766	0.1391	198769	141852	1.4012
C5	3.9733	0.1589	228745	142524	1.605
Slope					**10.4350**
Intercept					**-0.0470**
CC					**0.9999**

Representative Chromatogram of Analytical standard C1

Representative Chromatogram of Batch 1

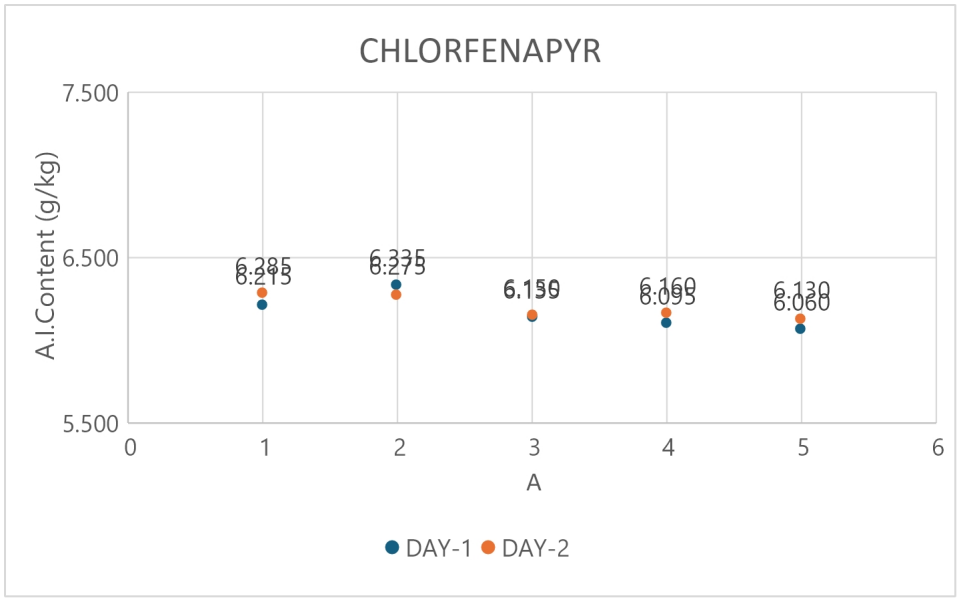


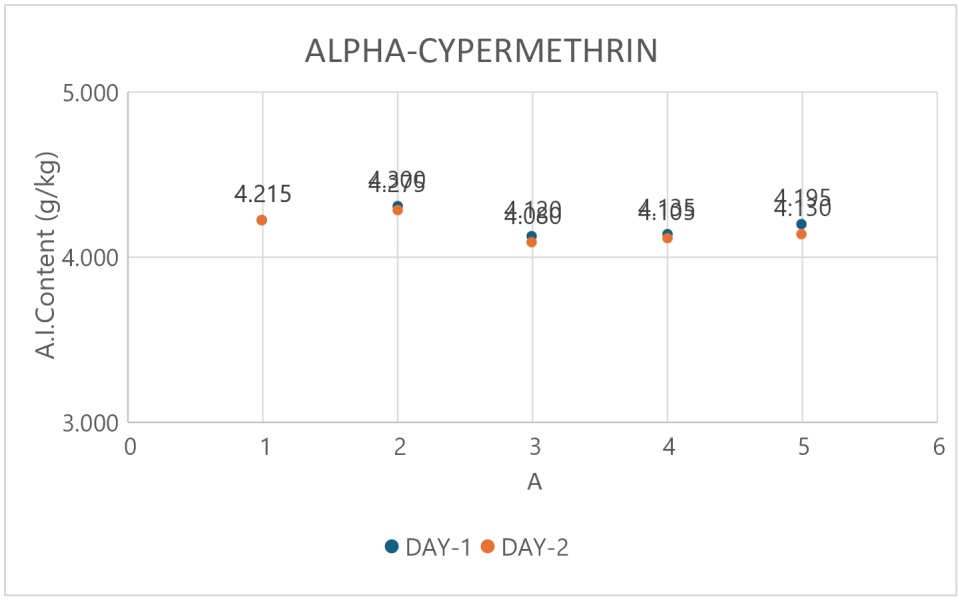
CHLORFENAPYR

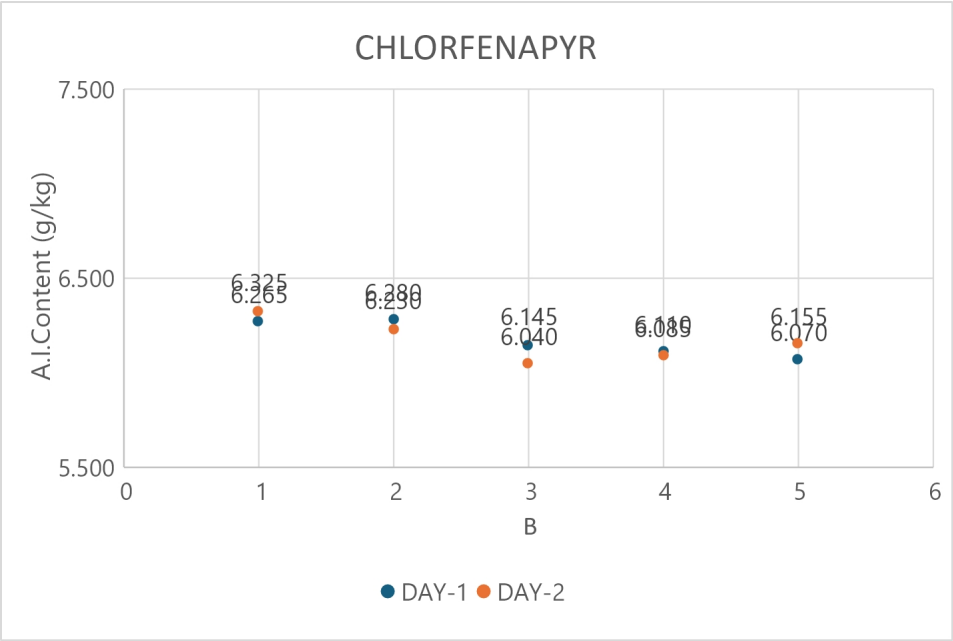
	DAY-1		DAY-2		MEAN	SD	%RSD	%RSDr	Horrat value
1A	6.2	6.23	6.23	6.34	6.25	0.0616	0.99	2.88	0.34
1B	6.26	6.27	6.25	6.4	6.30	0.0705	1.12	2.87	0.39
2A	6.32	6.35	6.33	6.22	6.31	0.0580	0.92	2.87	0.32
2B	6.26	6.3	6.31	6.15	6.26	0.0733	1.17	2.88	0.41
3A	6.15	6.12	6.08	6.22	6.14	0.0591	0.96	2.88	0.33
3B	6.13	6.16	6.02	6.06	6.09	0.0640	1.05	2.89	0.36
4A	6.14	6.05	6.14	6.18	6.13	0.0550	0.90	2.88	0.31
4B	6.02	6.2	6.07	6.1	6.10	0.0759	1.24	2.89	0.43
5A	6.03	6.09	6.11	6.15	6.10	0.0500	0.82	2.89	0.28
5B	6.01	6.13	6.14	6.17	6.11	0.0704	1.15	2.89	0.40

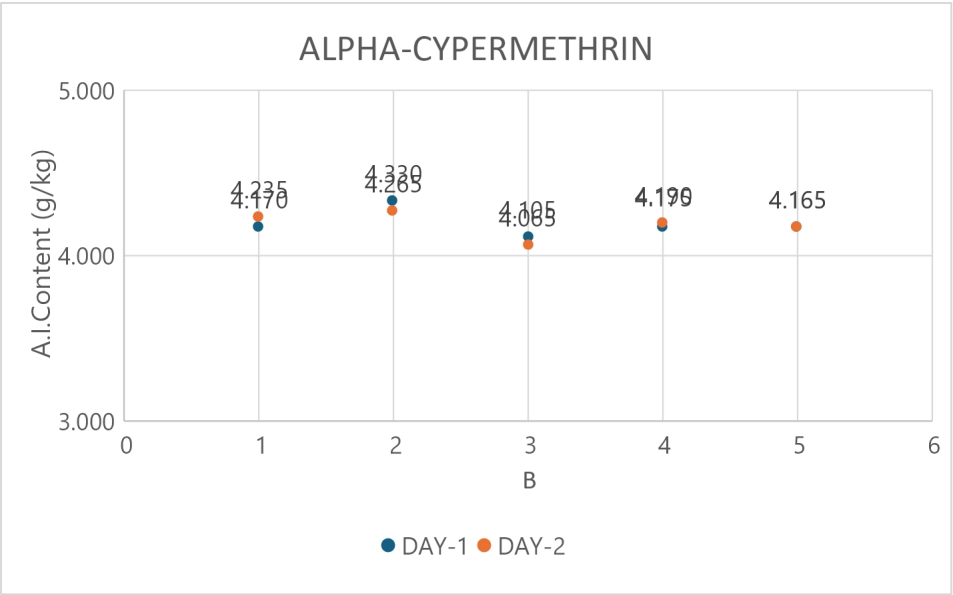
ALPHA-CYPERMETHRIN

	DAY-1		DAY-2		MEAN	SD	%RSD	%RSDr	Horrat value
1A	4.23	4.2	4.2	4.23	4.22	0.0173	0.41	3.05	0.13
1B	4.19	4.15	4.25	4.22	4.20	0.0427	1.02	3.05	0.33
2A	4.27	4.33	4.26	4.29	4.29	0.0310	0.72	3.04	0.24
2B	4.32	4.34	4.26	4.27	4.30	0.0386	0.90	3.04	0.30
3A	4.13	4.11	4.06	4.1	4.10	0.0294	0.72	3.06	0.24
3B	4.07	4.14	4.11	4.02	4.09	0.0520	1.27	3.07	0.41
4A	4.17	4.1	4.1	4.11	4.12	0.0337	0.82	3.06	0.27
4B	4.16	4.19	4.24	4.14	4.18	0.0435	1.04	3.06	0.34
5A	4.19	4.2	4.1	4.16	4.16	0.0450	1.08	3.06	0.35
5B	4.15	4.18	4.14	4.19	4.17	0.0238	0.57	3.06	0.19


CHLORFENAPYR
7.500
6.500
5.500
A.I.Content (g/kg)
0
1
2
3
4
5
6
A
6.385
6.215
6.335
6.275
6.160
6.095
6.130
6.060
DAY-1
DAY-2


ALPHA-CYPERMETHRIN
5.000
4.000
3.000
A.I.Content (g/kg)
0
1
2
3
4
5
6
A
4.215
4.300
4.275
4.195
4.130
DAY-1
DAY-2





Lab 1 - **Dr. Ch. Rajasekharam , TFM**

NACL Industries Limited (A GLP Test facility)
Anthireddyguda road, Nandigaon Mandal
Kothur, Ranga Reddy District, Telangana, India.-509228

Lab 2 – Dr. A Ramesh , TFM
International Institute of Biotechnology and Toxicology, IIBAT,
(A GLP Test facility), Padappai, India 601301

Lab 1 and Lab 2 reported no changes in the method

Conclusions:

Five different batches were analyzed in two different laboratories in India.

The repeatability was established through the determination of Standard deviation

which is - <0.1 among 5 different batches, between the two labs tested and for the two actives Alphacypermethrin and Chlorfenapyr.

The HarRot value is well within <1.

The repeatability data clearly reflects the method is well suitable for the extension of scope with minor modifications of temperature.

The method extension may please be considered and adopted as extension method for Terminator