Piperonyl Butoxide

33

CIPAC 33/LN/(M)/3

Method extension

Studies for Method Extension of existing CIPAC method for Piperonyl Butoxide

Long Lasting Insecticidal Nets.

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1. **Introduction**

- The CIPAC method 33/LN/(M) is suitable for determining piperonyl butoxide (PBO) impregnated insecticidal nets in the presence of deltamethrin. This report was prepared to demonstrate CIPAC 33/LN/(M) also suitable for determining piperonyl butoxide in coated insecticidal nets in the presence of deltamethrin.

  The study is method extension of CIPAC method 33/LN/(M) for the evaluation of Piperonyl Butoxide (PBO) in Yorkool G4 long lasting insecticidal nets. The study was conducted by Yorkool International Trading Co., Ltd.

2. **Method of CIPAC 33/LN/(M)**

Outline of CIPAC Method: The sample is extracted by refluxing with acetone. The piperonyl butoxide content is determined by capillary gas chromatography using flame ionisation detection and internal standard

Reagents

Piperonyl butoxide standard of known purity. Store below 0°C

Octadecane internal standard

Acetone

Internal standard solution. Weigh(to the nearest 0. 1 mg) into a volumetric flask 50 ml)octadecane(0. 4 g). Fill to the mark with acetone and mix well

Calibration solutions. Allow piperonyl butoxide to equilibrate to ambient temperature. Then weigh(to the nearest 0.1 mg) 0. 25 g piperonyl butoxide (s mg) into a volumetric flask(50 ml). Fill to the mark with acetone and mix well. Transfer using a graduated pipette 0.50 ml, 1.50 ml, 2.00 ml, 3.00 ml and 4.00 ml of this solution to 5 volumetric flasks(25 ml). Add internal standard solution(2.0 ml)to each flask, fill each to the mark with acetone and mix well

(solutions CA, CB, CC, CD, and CE). Filter the solutions through a 0.45 μm PTFE filter membrane and transfer 200 ul portions into GC vials

Apparatus

Gas chromatograph capable of operating over the range of 180 to 250°C, fit with flame ionisation detection

Capillary column fused silica, 30 m X 0.32 mm(i.d.)coated with 100 methyl polysiloxane, cross-linked, surface bonded stationary phase and 0.25µm film thickness (TRI or equivalent)

Hot plate with magnetic stirrer

Reflux condenser

Disposable syringe with 0.45µm filter

Electronic integrator or data *system*

Procedure

a. *Operating conditions(typical)*

Column Fused silica, 30 m x 0.32 mm (i.d.) with 100% methyl polysiloxane, cross-linked, surface bonded stationery phase and 0. 25µm film thickness (Durabond-I or equivalent)

Injection system

Injector	Split injection
Injector temperature	250°C
Split ratio	30:1
Purge flow	1 ml/min
Injection volume	1 µl

Detector system

Type	Flame ionisation
Temperature	300°C

Oven temperatures

Initial	180°C
Program	180°C hold for 11 min
	210°C at 10°Cmin, hold for8min

	220°C at 10°C/min. hold for 18 min
	245°C at 30°C/min, hold for 4 min
Total run time 45 min	

Gas fow rates

Helium(carrier)	linear velocity: 39 cm/sec at 180°C
Helium(make up)	30 ml/ min
Hydrogen	40 ml/min
Air	400 ml/min
Total flow	35ml/min
Retention times	piperonyl butoxide: about 22.1 min
	Octadecane: about 6.2 min

b. *Preparation of sample.*
Cut the sample into small pieces of less than 2.2 cm and homogenise Weigh(to the nearest 0. 1 mg)about 0.5 g(w g) of the sample into a reflux flask(100 ml). Add acetone(23.0 ml) and by pipette internal standard solution (2.0 ml). Attach the flask to the reflux condenser and reflux the sample for about 30 min while stirring. Cool the sample to room temperature and lilter the solution through a 0.45µm PTFE filter membrane. Transfer 200 µl of the sample to a GC vial.

c. *System equilibration*
Inject into the gas chromatograph a 1 µl portion of the sample solution to condition the column and to check for the appropriate flow rates and integration events.

d. *Determination and preparation of calibration curve*
Inject in duplicate into the gas chromatograph 1 µl portions of calibration solutions and sample solutions in the following sequence:

CA, CA, CB, CB, CC, CC, CD, CD, CE, CE, S1, S1, S2, S2… etc

Prepare a curve by plotting the piperonyl butoxide to internal standard peak area ratios versus the mass of piperonyl butoxide in the calibration solutions(0.01s, 0.03s, 0.04s, 0.06s, and 0.08s mg respectively). Using the method of least squares calculate the equation for the straight line that best fits the experimental data. The correlation coefficient should be 0.999 or better. Determine the piperonyl butoxide to internal

standard response ratios of the sample solutions and calculate the average(R) for each sample.

e. *Calculation*
Content of piperonyl butoxide= $\frac{(R - b) * P}{a * w} g / kg$

where:

R=average piperonyl butoxide to octadecane peak area ratio in the sample

a=slope of calibration curve

b=intercept of calibration curve

P=purity of the piperonyl butoxide standard (g/kg)

Note1 A deltamethrin peak will not be visible on the chromatogram at the conditions given.

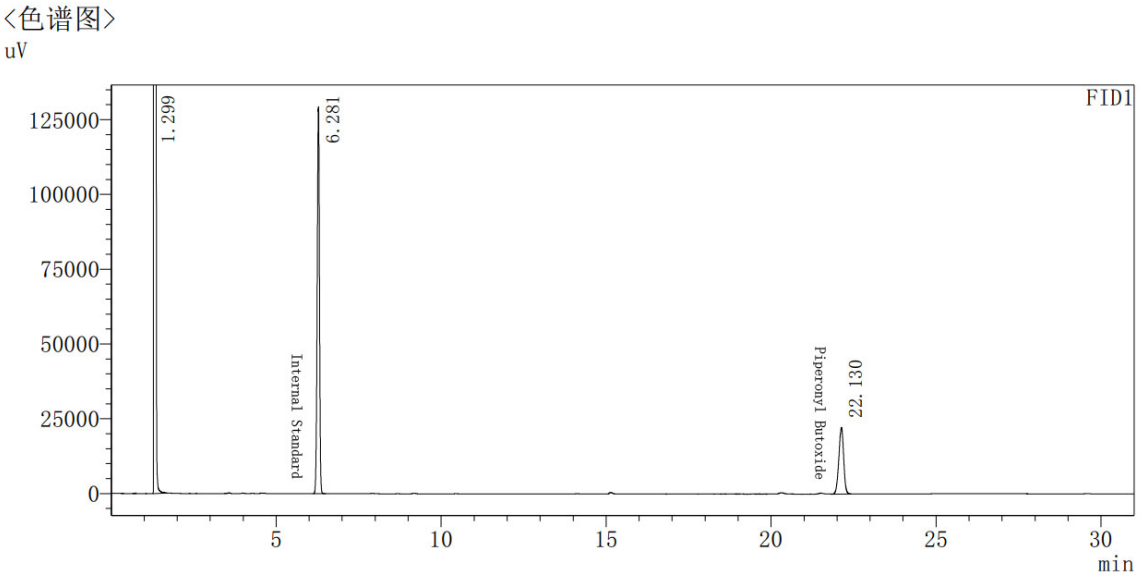


Figure 1 GC Chromatogram of a calibration solution

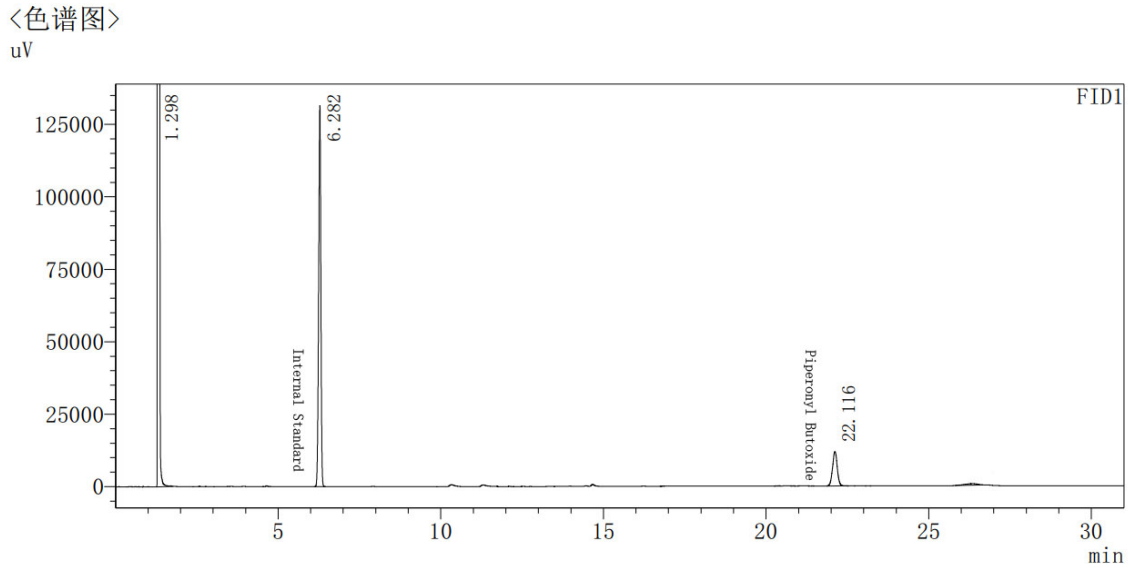


Figure 2 GC Chromatogram of a sample solution

3. **Method Assessment**

According to the CIPAC method extension guideline, the method extension of the CIPAC 33/LN/(M) - for piperonyl butoxide in coated insecticidal nets in the presence of deltamethrin.

Check the availability of a CIPAC method for the formulation concerned (Step 1)

The formulation is long lasting insecticidal nets. There is existing CIPAC method available for the long lasting insecticidal nets.

Check whether the concentration of the analyte is inside or outside the acceptability range covered by the samples of the original trial (Step 2)

CIPAC 33/LN/(M)/3 was evaluated for concentrations between 100 µg/ml and 800µg/ml, the sample concentration is 440± 25% µg/ml (between 330 µg/ml and 550 µg/ml), and the concentration of the analyte is inside the acceptability range.

4. Results

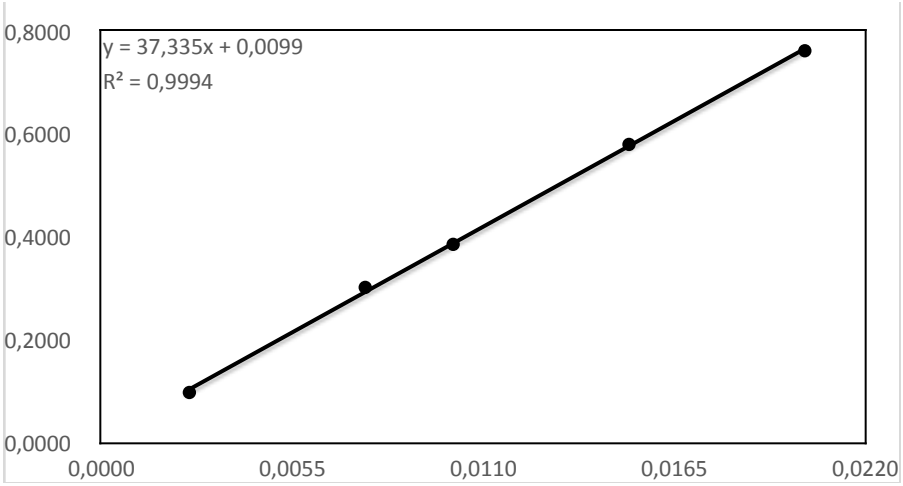
Day 1 results from *Test Center for Chemical Products of Zhejiang Chemical Industry Research Institute*

	Weight(g)
PBO neeting subsample1 (S1)	0.5126
PBO neeting subsample2 (S2)	0.5016
PBO neeting subsample3 (S3)	0.5120
PBO neeting subsample4 (S4)	0.5038
PBO neeting subsample5 (S5)	0.5192

purity of the standard PBO(g/kg)	976
weight of standard PBO(mg)	0.2532

Sample	peak area of octadecane	peak area of PBO	PBO to internal standard peak area ratios in the caliration	average PBO to internal standard peak area ratios in the caliration	mass of PBO in the caliration solutions(g)
calibration solution CA	380.85	37.50	0.0985	0.0983	0.0025
calibration solution CA	384.55	37.74	0.0981		
calibration solution CB	384.63	116.44	0.3027	0.3025	0.0076
calibration solution CB	384.92	116.33	0.3022		
calibration solution CC	386.26	149.10	0.3860	0.3861	0.0101
calibration solution CC	388.25	149.95	0.3862		
calibration solution CD	386.86	224.60	0.5806	0.5803	0.0152
calibration solution CD	387.46	224.72	0.5800		
calibration solution CE	385.24	293.36	0.7615	0.7622	0.0203
calibration solution CE	377.86	288.30	0.7630		
sample solution S1-1	314.75	69.17	0.2198	0.2203	/
sample solution S1-2	335.68	74.11	0.2208		
sample solution S2-1	354.62	75.36	0.2125	0.2120	/
sample solution S2-2	356.53	75.40	0.2115		
sample solution S3-1	387.72	83.30	0.2148	0.2149	/
sample solution S3-2	379.85	81.63	0.2149		
sample solution S4-1	349.52	74.35	0.2127	0.2128	/
sample solution S4-2	365.45	77.79	0.2129		
sample solution S5-1	360.25	80.99	0.2248	0.2242	/
sample solution S5-2	345.81	77.33	0.2236		

Curve by plotting the average PBO to internal standard peak area ratios versus the mass of PBO in the caliration solutions.



slope of calebration curve	37.3354
intercept of calibration curve	9.90E-03
purty of the PBO standard(g/kg)	976
content of PBO(g/kg) in sample1-1	10.70
content of PBO(g/kg) in sample1-2	10.75
content of PBO(g/kg) in sample2-1	10.56
content of PBO(g/kg) in sample2-1	10.51
content of PBO(g/kg) in sample3-1	10.46
content of PBO(g/kg) in sample3-2	10.47
content of PBO(g/kg) in sample4-1	10.52
content of PBO(g/kg) in sample4-2	10.53
content of PBO(g/kg) in sample5-1	10.82
content of PBO(g/kg) in sample5-2	10.76

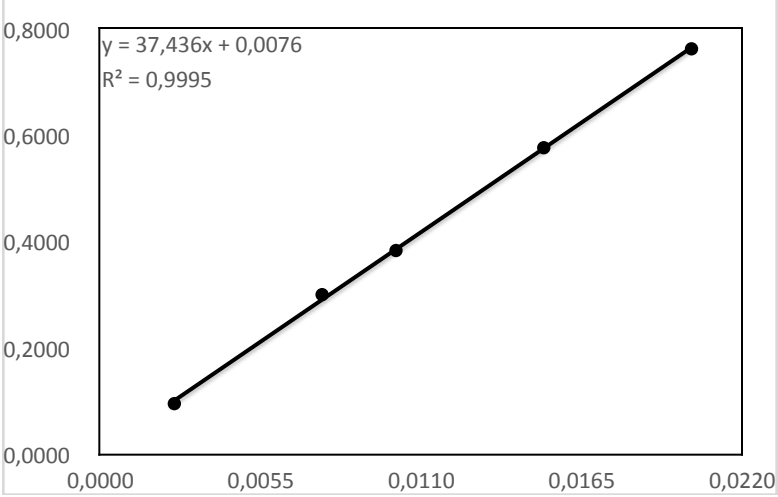
Day 2 results from *Test Center for Chemical Products of Zhejiang Chemical Industry Research Institute*

	Weight(g)
PBO neeting subsample1 (S1)	0.5103
PBO neeting subsample2 (S2)	0.5034
PBO neeting subsample3 (S3)	0.5100
PBO neeting subsample4 (S4)	0.5123
PBO neeting subsample5 (S5)	0.5039

purity of the standard PBO(g/kg)	976
weight of standard PBO(mg)	0.2532

Sample	peak area of octadecane	peak area of PBO	PBO to internal standard peak area ratios in the caliration solutions	average PBO to internal standard peak area ratios in the caliration solutions
calibration solution CA	441.44	42.68	0.0967	0.0966
calibration solution CA	443.84	42.88	0.0966	
calibration solution CB	436.28	131.40	0.3012	0.3014
calibration solution CB	358.94	108.28	0.3017	
calibration solution CC	472.16	181.36	0.3841	0.3843
calibration solution CC	468.86	180.32	0.3846	
calibration solution CD	452.33	260.97	0.5769	0.5775
calibration solution CD	453.18	261.97	0.5781	
calibration solution CE	429.16	327.87	0.7640	0.7634
calibration solution CE	422.92	322.58	0.7627	
sample solution S1-1	388.26	85.62	0.2205	0.2197
sample solution S1-2	386.68	84.60	0.2188	
sample solution S2-1	431.86	91.25	0.2113	0.2114
sample solution S2-2	489.18	103.42	0.2114	
sample solution S3-1	447.12	95.98	0.2147	0.2152
sample solution S3-2	451.52	97.45	0.2158	
sample solution S4-1	426.86	94.97	0.2225	0.2225
sample solution S4-2	424.60	94.46	0.2225	
sample solution S5-1	421.28	88.96	0.2112	0.2110
sample solution S5-2	417.30	88.01	0.2109	

Curve by plotting the average PBO to internal standard peak area ratios versus the mass of PBO in the caliration solutions.



slope of calebration curve	37.4357
intercept of calibration curve	7.60E-03
purty of the PBO standard(g/kg)	976
content of PBO(g/kg) in sample1-1	10.88
content of PBO(g/kg) in sample1-2	10.79
content of PBO(g/kg) in sample2-1	10.55
content of PBO(g/kg) in sample2-1	10.56
content of PBO(g/kg) in sample3-1	10.59
content of PBO(g/kg) in sample3-2	10.64
content of PBO(g/kg) in sample4-1	10.94
content of PBO(g/kg) in sample4-2	10.93
content of PBO(g/kg) in sample5-1	10.53
content of PBO(g/kg) in sample5-2	10.52

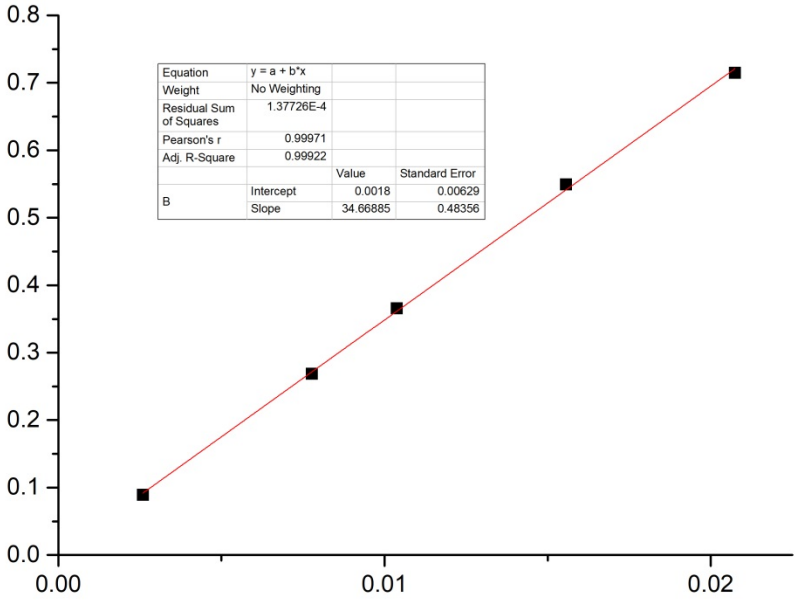
Day 1 results from *Tianjin Yorkool International Trading Co., Ltd.*

	Weight(g)
PBO neeting subsample1 (S1)	0.5001
PBO neeting subsample2 (S2)	0.4985
PBO neeting subsample3 (S3)	0.4962
PBO neeting subsample4 (S4)	0.4945
PBO neeting subsample5 (S5)	0.4964

purity of the standard PBO(g/kg)	976
weight of standard PBO(mg)	0.2593

Sample	peak area of octadecane	peak area of PBO	PBO to internal standard peak area ratios in the caliration	average PBO to internal standard peak area ratios in the caliration	mass of PBO in the caliration solutions(g)
calibration solution CA	571828	50923	0.0891	0.0892	0.0026
calibration solution CA	588063	52582	0.0894		
calibration solution CB	584311	157066	0.2688	0.2685	0.0078
calibration solution CB	612340	164221	0.2682		
calibration solution CC	617466	225127	0.3646	0.3655	0.0104
calibration solution CC	596260	218456	0.3664		
calibration solution CD	566884	311178	0.5489	0.5491	0.0156
calibration solution CD	598754	328888	0.5493		
calibration solution CE	626759	445955	0.7115	0.7144	0.0207
calibration solution CE	613022	439721	0.7173		
sample solution S1-1	666884	122317	0.1834	0.1834	/
sample solution S1-2	642380	117827	0.1834		
sample solution S2-1	653755	119015	0.1820	0.1829	/
sample solution S2-2	622872	114455	0.1838		
sample solution S3-1	640008	114034	0.1782	0.1783	/
sample solution S3-2	642260	114553	0.1784		
sample solution S4-1	616307	117359	0.1904	0.1902	/
sample solution S4-2	632133	120138	0.1901		
sample solution S5-1	635623	121480	0.1911	0.1905	/
sample solution S5-2	649651	123362	0.1899		

Curve by plotting the average PBO to internal standard peak area ratios versus the mass of PBO in the caliration solutions.



slope of calebration curve	34.66885
intercept of calibration curve	1.80E-03
purty of the PBO standard(g/kg)	976
content of PBO(g/kg) in sample1-1	10.31
content of PBO(g/kg) in sample1-2	10.32
content of PBO(g/kg) in sample2-1	10.27
content of PBO(g/kg) in sample2-1	10.37
content of PBO(g/kg) in sample3-1	10.10
content of PBO(g/kg) in sample3-2	10.11
content of PBO(g/kg) in sample4-1	10.83
content of PBO(g/kg) in sample4-2	10.81
content of PBO(g/kg) in sample5-1	10.83
content of PBO(g/kg) in sample5-2	10.76

Day 2 results from *Tianjin Yorkool International Trading Co., Ltd.*

	Weight(g)

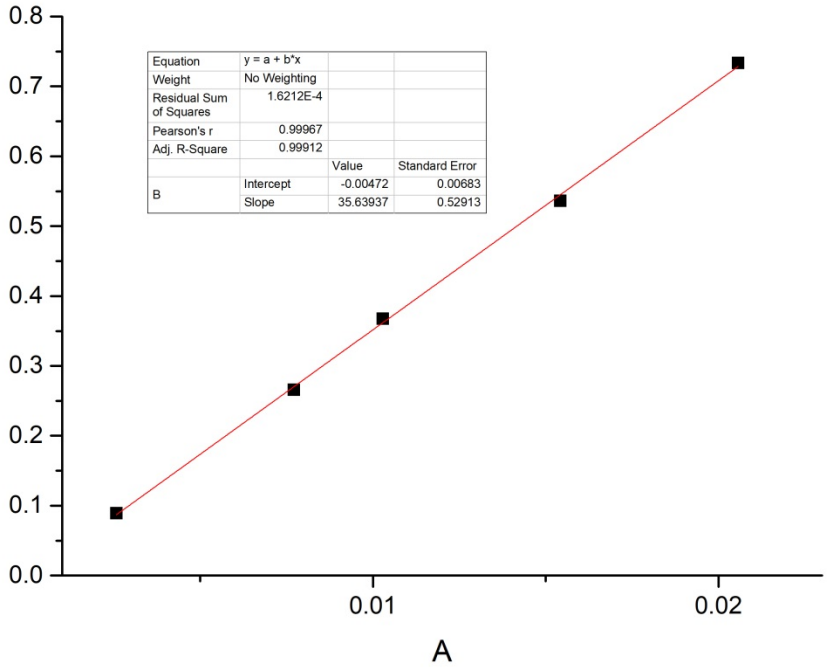
PBO neeting subsample1 (S1)	0.5054
PBO neeting subsample2 (S2)	0.5031
PBO neeting subsample3 (S3)	0.5044
PBO neeting subsample4 (S4)	0.4935
PBO neeting subsample5 (S5)	0.4988

purity of the standard PBO(g/kg)	976
weight of standard PBO(mg)	0.2571

Sample	peak area of octadecane	peak area of PBO	PBO to internal standard peak area ratios in the caliration	average PBO to internal standard peak area ratios in the caliration	mass of PBO in the caliration solutions(g)
calibration solution CA	562333	49816	0.0886	0.0893	0.0026
calibration solution CA	580509	52205	0.0899		
calibration solution CB	589672	156345	0.2651	0.2655	0.0077
calibration solution CB	579582	154146	0.2660		
calibration solution CC	577584	212297	0.3676	0.3678	0.0103
calibration solution CC	597228	219787	0.3680		
calibration solution CD	628650	336163	0.5347	0.5364	0.0154
calibration solution CD	621254	334264	0.5380		
calibration solution CE	608042	446522	0.7344	0.7332	0.0206
calibration solution CE	616445	451323	0.7321		
sample solution S1-1	612807	111884	0.1826	0.1831	/
sample solution S1-2	639017	117293	0.1836		
sample solution S2-1	639924	116550	0.1821	0.1828	/
sample solution S2-2	636361	116731	0.1834		
sample solution S3-1	671572	123518	0.1839	0.1849	/
sample solution S3-2	671778	124921	0.1860		
sample solution S4-1	641055	122409	0.1909	0.1915	/
sample solution S4-2	621888	119476	0.1921		

sample solution S5-1	630299	119950	0.1903	0.1909	/
sample solution S5-2	610348	116876	0.1915		

Curve by plotting the average PBO to internal standard peak area ratios versus the mass of PBO in the caliration solutions.



slope of calebration curve	35.63937
intercept of calibration curve	-4.72E-03
purty of the PBO standard(g/kg)	976
content of PBO(g/kg) in sample1-1	10.16
content of PBO(g/kg) in sample1-2	10.21
content of PBO(g/kg) in sample2-1	10.18
content of PBO(g/kg) in sample2-1	10.25
content of PBO(g/kg) in sample3-1	10.26
content of PBO(g/kg) in sample3-2	10.37
content of PBO(g/kg) in sample4-1	10.88
content of PBO(g/kg) in sample4-2	10.95

content of PBO(g/kg) in sample5-1	10.73
content of PBO(g/kg) in sample5-2	10.80

Summary of the results of two laboratories

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Lab 1	Day 1	10.70	10.56	10.46	10.52	10.82
	Day 1	10.75	10.51	10.47	10.53	10.76
	Day 2	10.88	10.55	10.59	10.94	10.53
	Day 2	10.79	10.56	10.64	10.93	10.52
	STD	0.074	0.025	0.090	0.235	0.155
	Average	10.78	10.54	10.54	10.73	10.66
Lab 2	Day 1	10.31	10.27	10.10	10.83	10.83
	Day 1	10.32	10.37	10.11	10.81	10.76
	Day 2	10.16	10.18	10.26	10.88	10.73
	Day 2	10.21	10.25	10.37	10.95	10.80
	STD	0.077	0.076	0.129	0.062	0.043
	Average	10.25	10.27	10.21	10.87	10.78
	Total Average	10.52	10.41	10.37	10.80	10.72

The following table is obtained by analyzing the test data.

	sample A	sample B	sample C	sample D	Sample E
x	10.52	10.41	10.37	10.80	10.72
L	2	2	2	2	2
S_r	0.08	0.06	0.11	0.17	0.11
S_L	0.37	0.19	0.23	0.04	0.06
S_R	0.38	0.20	0.25	0.18	0.13
RSD_r	0.72	0.55	1.07	1.59	1.06
RSD_R	3.62	1.92	2.45	1.64	1.22

r	0.21	0.16	0.31	0.48	0.32
R	1.07	0.56	0.71	0.50	0.37
RSD_R(Hor)	3.97	3.98	3.98	3.95	3.96
HorRat	0.91	0.48	0.62	0.42	0.31

Where:

X = average

L = number of laboratories

s_r = repeatability standard deviation

s_L = "pure" between laboratory standard variation

s_R = reproducibility standard deviation= $\sqrt{(s_r^2 + s_L^2)}$

RSD_r = repeatability relative standard deviation ($s_r/x*100$)

RSD_R = reproducibility relative standard deviation ($s_R/x*100$)

r = repeatability ($s_r*2.8$)

R = reproducibility ($s_R*2.8$)

RSD_R(Hor) = Horwitz value calculated from: $2^{(1-0.5\log c)}$

where c = the concentration of the analyte as a decimal fraction

HorRat = RSD_R/RSD_R(Hor)

Conclusion

In order to demonstrate CIPAC 33/LN/(M) also suitable for determining piperonyl butoxide in coated insecticidal nets in the presence of deltamethrin. Validation tests were conducted in two laboratories.

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In addition to the known criteria the use of the HorRat value could be an additional criterion.

$0.3 \leq$ HorRat ≤ 1 => fully acceptable

HorRat < 0.3 or $1 <$ HorRat ≤ 2 => Acceptable, but reasonable explanation required!

HorRat > 2 => not acceptable

The two laboratories data shown all sample's HorRat is between 0.3 and 1, so it demonstrates that the method is accuracy and repeatability, CIPAC method 33/LN/(M) can be extended in coated insecticidal nets.