



Determination of deltamethrin and/or piperonyl butoxide in Long-Lasting (incorporated into polyethylene) Insecticidal Mosquito Nets

Validation and performance verification of the analytical method by GC-FID

REPORT TO CIPAC

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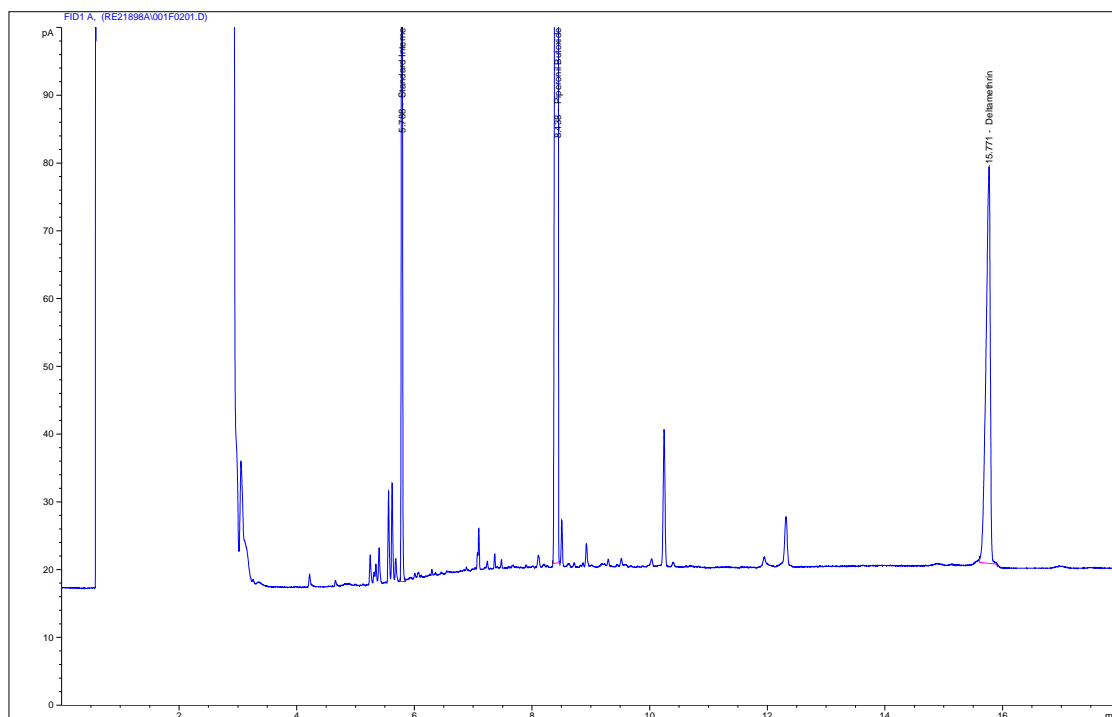
1. Validation of the analytical method

The validation of the analytical method for determination of deltamethrin and/or piperonyl butoxide in Long-Lasting (incorporated into polyethylene) Insecticidal Mosquito Nets (incorporated LNs) was performed on April 2008 in the framework of the CRA-W study 21678.

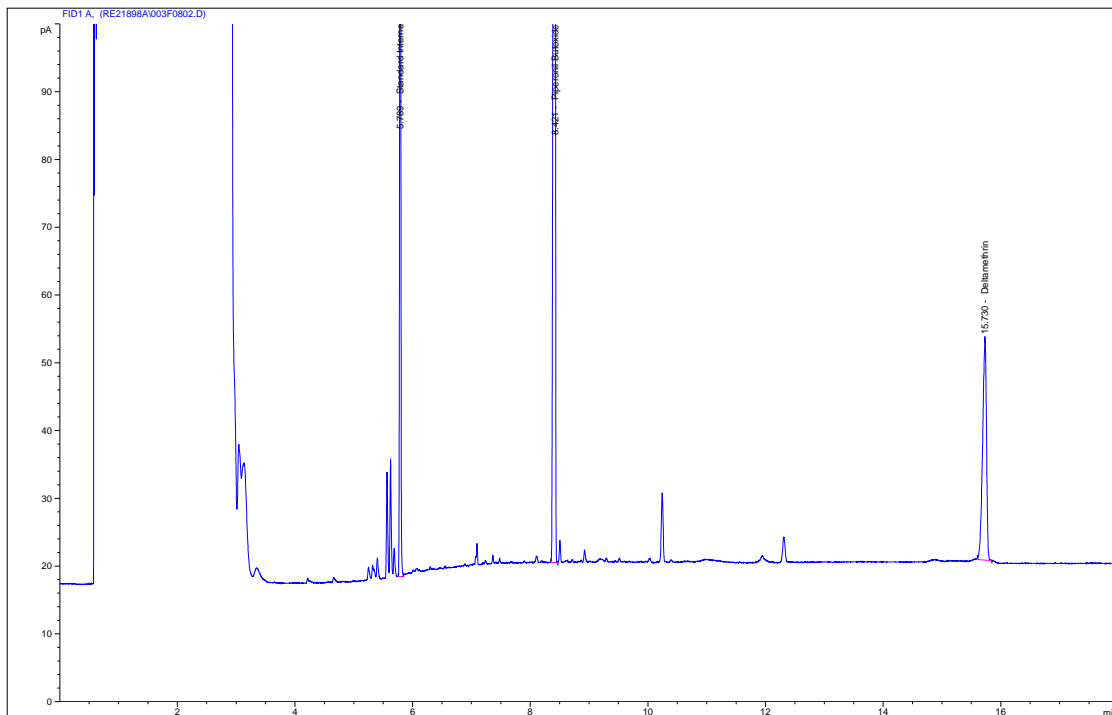
1.1 Specificity

The analysis of reagent blanks (method without sample) and untreated net samples in comparison with the analysis of standard solutions, spiked untreated net samples and treated net samples showed the absence of compound interfering with the determination of deltamethrin, deltamethrin R-isomer and piperonyl butoxide.

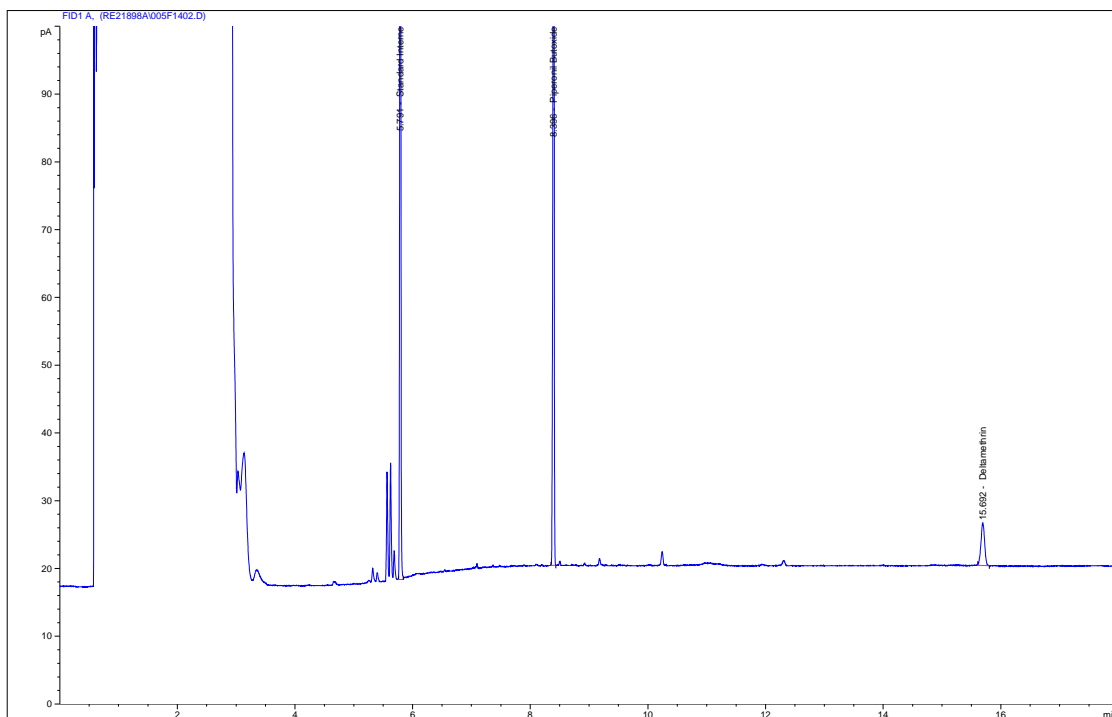
Representative chromatograms



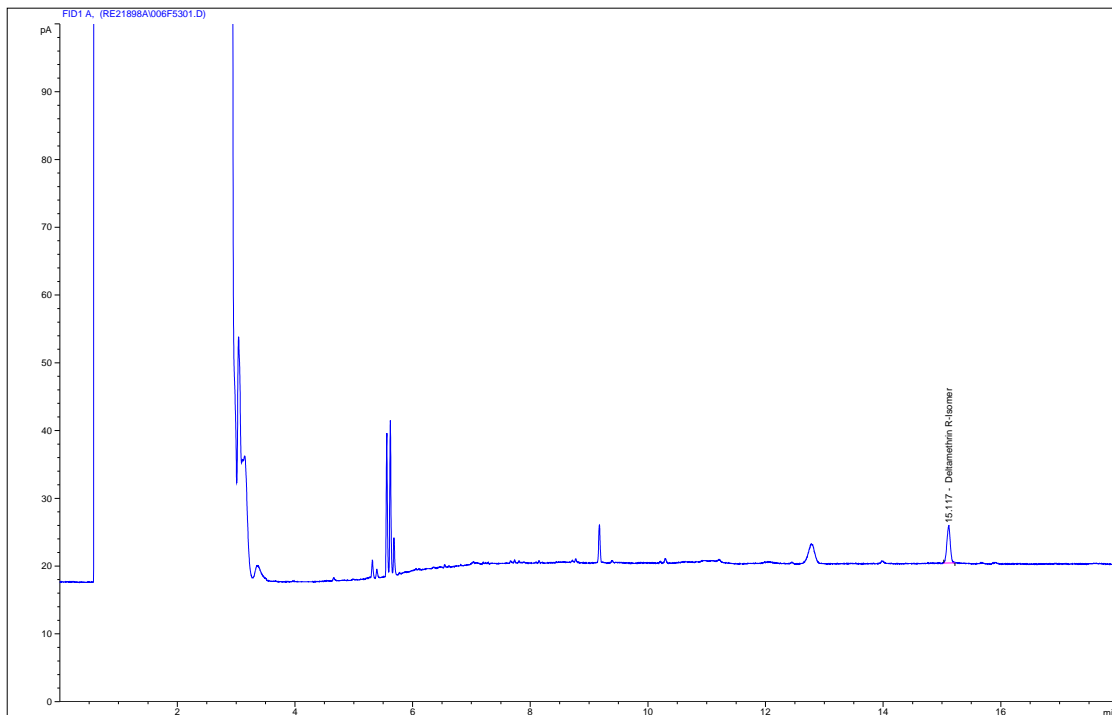
Deltamethrin and piperonyl butoxide calibration solution (C₅)



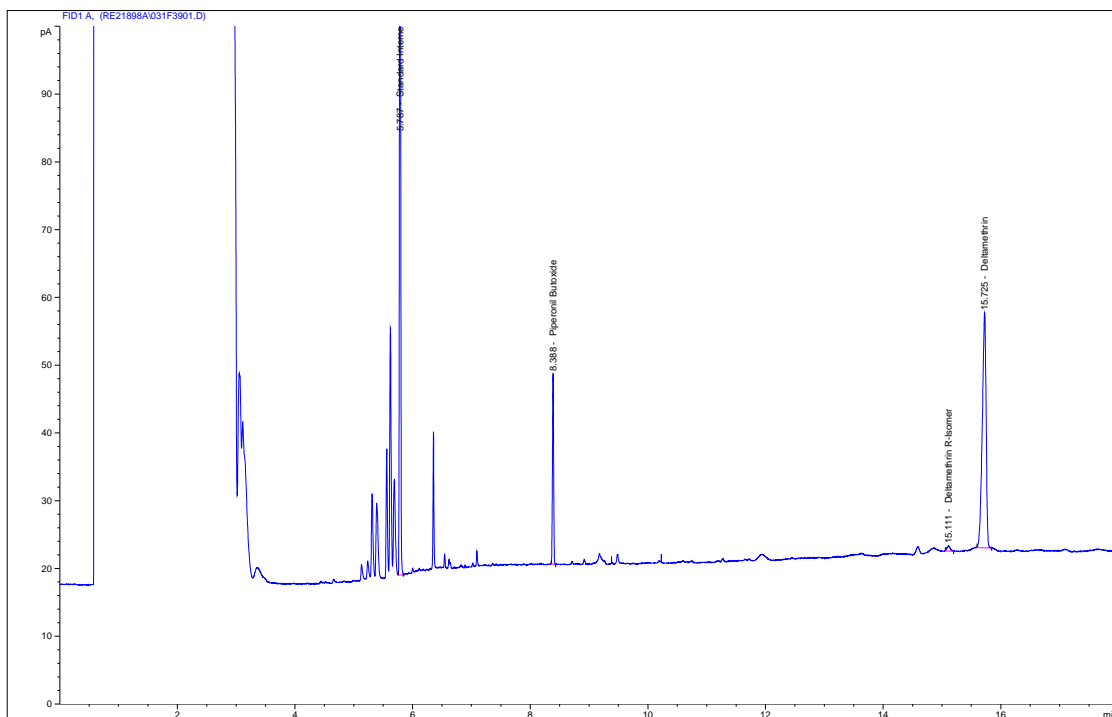
Deltamethrin and piperonyl butoxide calibration solution (C₃)



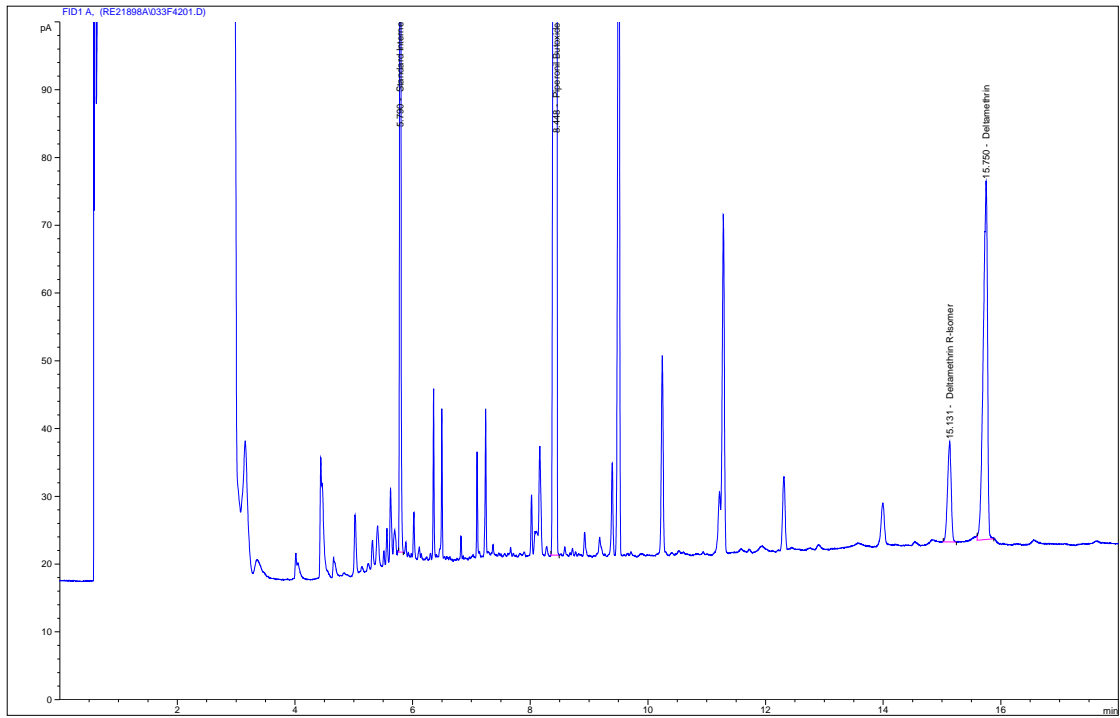
Deltamethrin and piperonyl butoxide calibration solution (C₁)



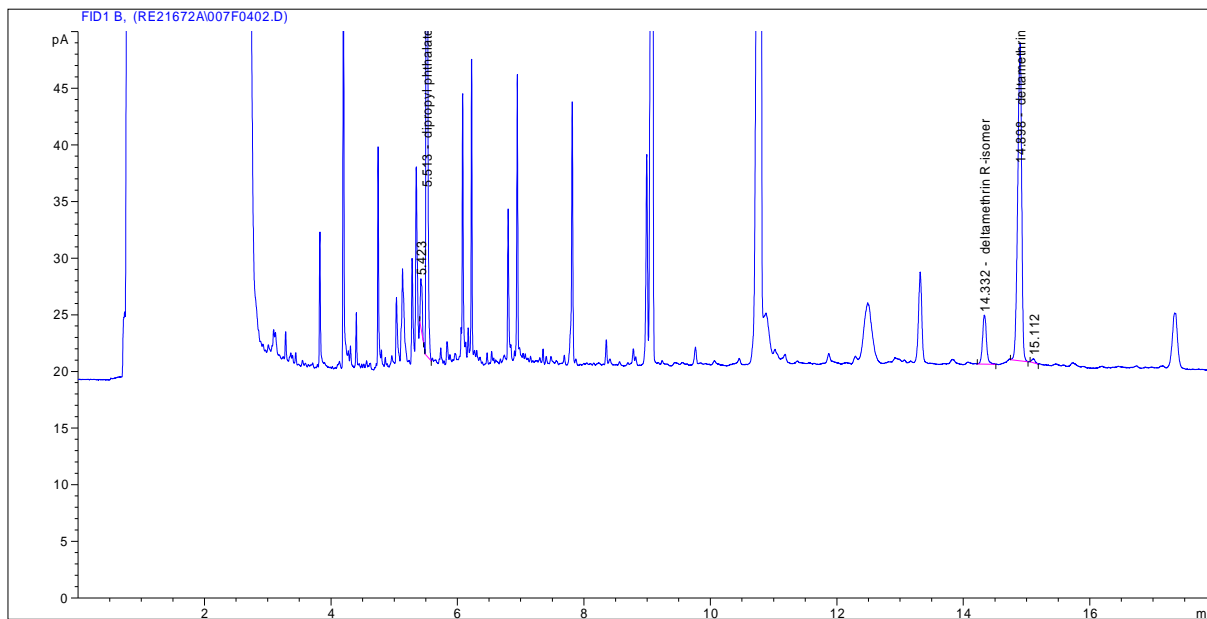
Deltamethrin R-isomer calibration solution



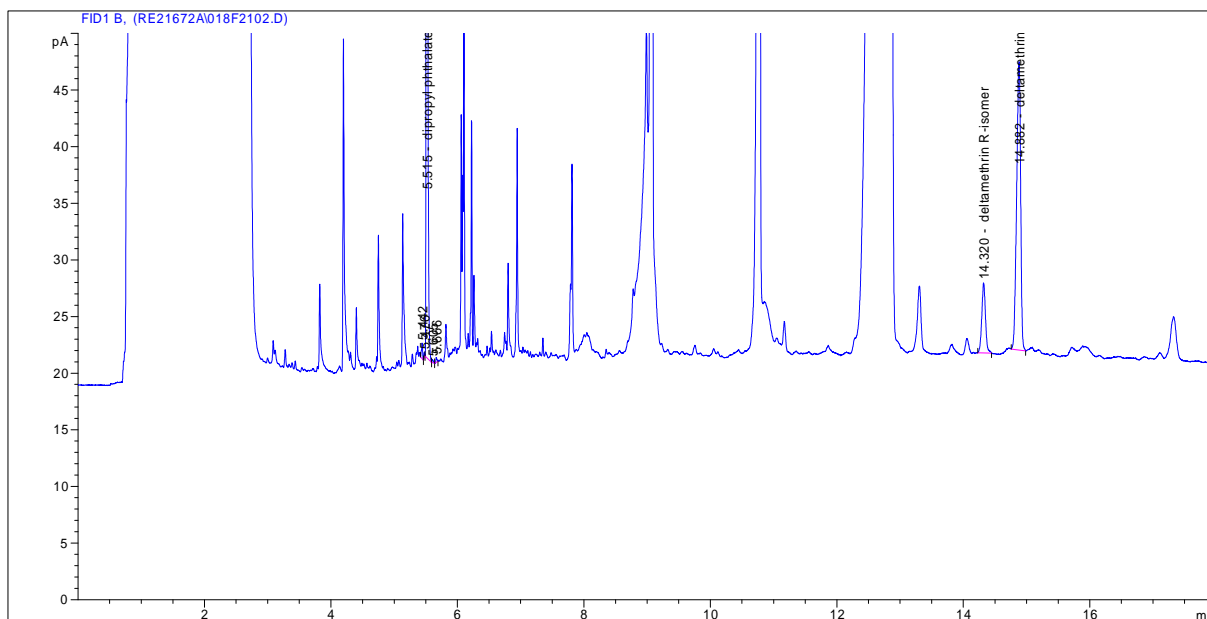
PermaNet 3.0® side sample solution (Re 16029/71)



PermaNet 3.0® roof sample solution (Re 16029/73)



NetProtect® sample solution (134 blue)

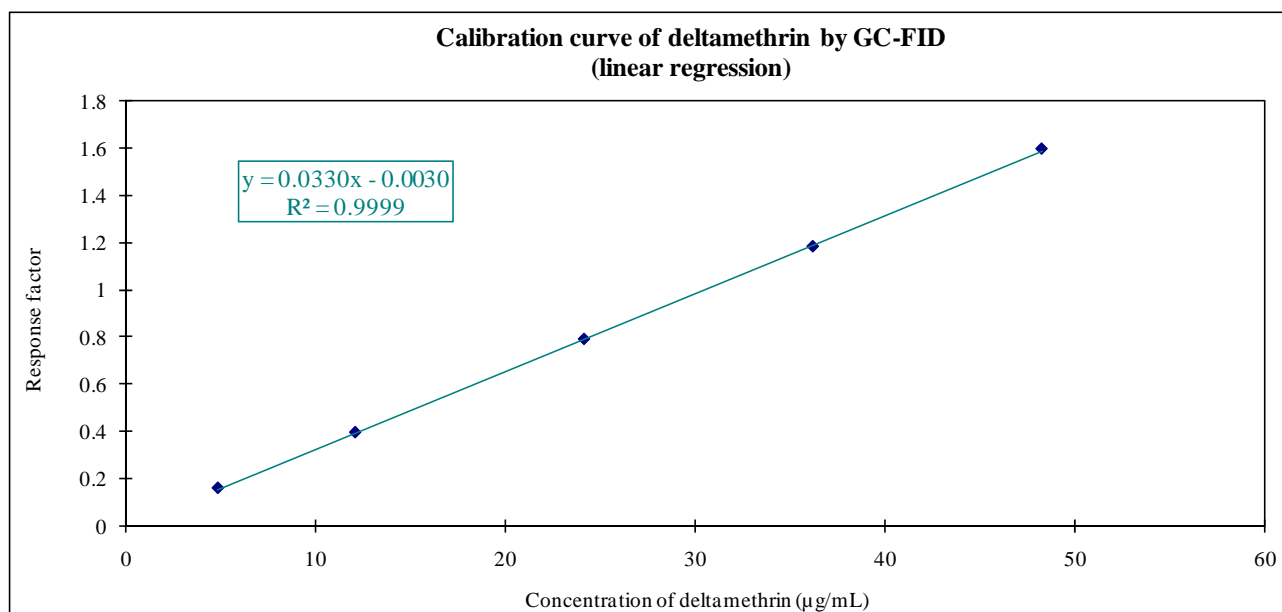


NetProtect® sample solution (158 white)

1.2 Linearity of the detector response

Deltamethrin

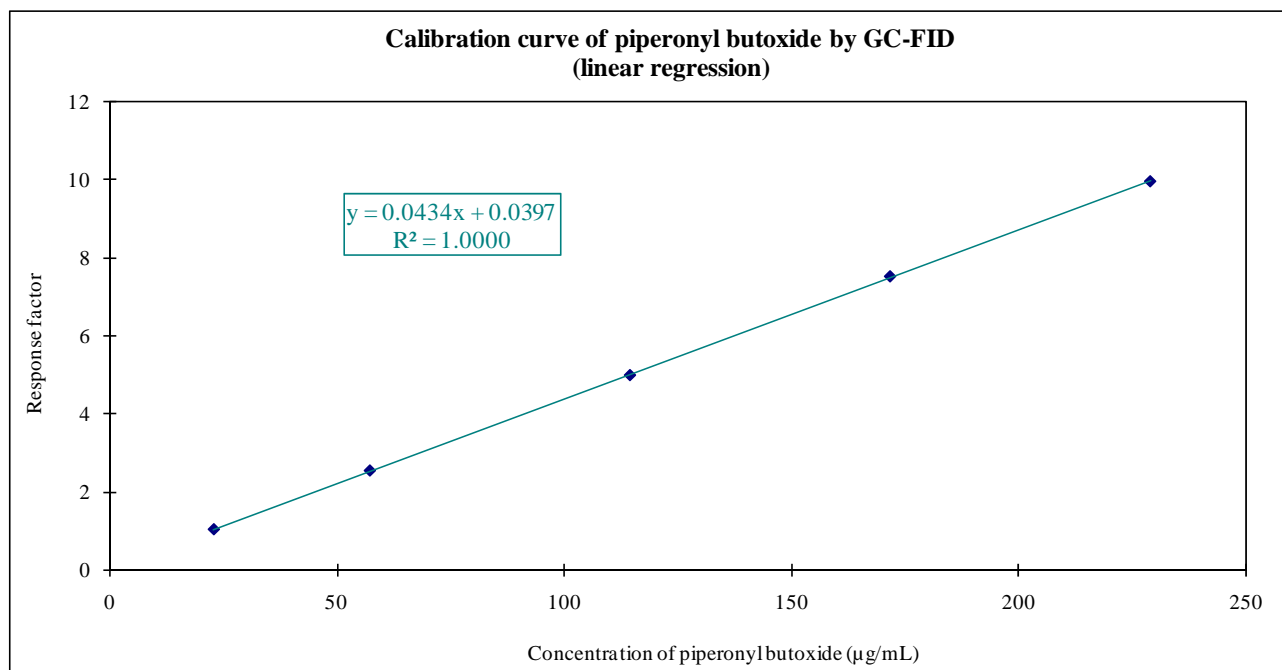
The linearity was demonstrated over a range of concentrations from 5 µg/mL to 50 µg/mL expressed as deltamethrin by measuring detector response factor (deltamethrin to internal standard area ratio) versus deltamethrin concentration for a series of 5 standard solutions of deltamethrin of 5, 12, 24, 36 and 50 µg/mL). Linear regression (least squares method) was calculated on the mean of minimum 2 injections of each standard solution.



The response factor (deltamethrin to internal standard area ratio) is linear in the range from 5 µg/mL to 50 µg/mL ($R^2 > 0.99$).

Piperonyl butoxide

The linearity was demonstrated over a range of concentrations from 24 $\mu\text{g/mL}$ to 240 $\mu\text{g/mL}$ expressed as piperonyl butoxide by measuring detector response factor (piperonyl butoxide to internal standard area ratio) versus piperonyl butoxide concentration for a series of 5 standard solutions of piperonyl butoxide of 24, 60, 120, 180 and 240 $\mu\text{g/mL}$. Linear regression (least squares method) was calculated on the mean of minimum 2 injections of each standard solution.



The response factor (piperonyl butoxide to internal standard area ratio) is linear in the range from 24 $\mu\text{g/mL}$ to 240 $\mu\text{g/mL}$ ($R^2 > 0.99$).

1.3 Repeatability

The repeatability of the method was assessed by performing 5 or 6 replicate determinations on 2 NetProtect samples.

Sample identification	Weighting number	Sample weight (g)	Deltamethrin content (g/kg)	Deltamethrin R-isomer content (g/kg)
134 blue	1	0.2994	1.55	0.248
134 blue	2	0.2998	1.59	0.265
134 blue	3	0.2997	1.56	0.259
134 blue	4	0.2996	1.50	0.260
134 blue	5	0.2997	1.54	0.265
Mean			1.55	0.259
SD			0.03	0.007
RSD			1.9%	2.7%
158 white	1	0.2995	1.51	0.358
158 white	2	0.3004	1.48	0.361
158 white	3	0.2999	1.52	0.362
158 white	4	0.2995	1.50	0.361
158 white	5	0.3000	1.50	0.367
158 white	6	0.2994	1.52	0.374
Mean			1.51	0.364
SD			0.01	0.006
RSD			0.9%	1.6%

The repeatability of the method is very good (RSD < 2% for deltamethrin content and < 3% for deltamethrin R-isomer content).

1.4 Accuracy and repeatability (recoveries)

Recovery values were calculated by fortifying untreated net samples with known amounts of deltamethrin and piperonyl butoxide. The untreated net sample was analysed prior to spiking and showed the absence of compound interfering with determination of deltamethrin and piperonyl butoxide (< 30 % LOQ).

Deltamethrin

Sample	Fortification level (g/kg as deltamethrin)	Extraction date	Injection date	Recovery (*) (%)	
Mosquito net	0.398	April 2008	April 2008	85	
	0.399	April 2008	April 2008	92	
	0.399	April 2008	April 2008	95	
	0.403	April 2008	April 2008	94	
	0.403	April 2008	April 2008	98	
	Mean				93
	Standard deviation				5
	Relative standard deviation				5.2 %
	Confidence interval (**)				93 ± 6
	1.99	April 2008	April 2008	92	
	1.99	April 2008	April 2008	97	
	1.99	April 2008	April 2008	96	
	2.01	April 2008	April 2008	95	
	2.00	April 2008	April 2008	98	
	Mean				96
	Standard deviation				2
	Relative standard deviation				2.4 %
	Confidence interval (**)				96 ± 3
	3.97	April 2008	April 2008	99	
	3.98	April 2008	April 2008	97	
	3.97	April 2008	April 2008	98	
	4.01	April 2008	April 2008	92	
	4.02	April 2008	April 2008	98	
	Mean				97
Standard deviation				3	
Relative standard deviation				2.9 %	
Confidence interval (**)				97 ± 3	
Mean				95	
Standard deviation				4	
Relative standard deviation				3.9 %	
Confidence interval (**)				95 ± 2	

(*) Each result is the mean of 2 chromatographic injections.

(**) Student t-test with a probability of 95 %.

The average recovery for deltamethrin on mosquito net for each fortification level is in the range 90-110 % with a RSD < 5 %.

Piperonyl butoxide

Sample	Fortification level (g/kg as piperonyl butoxide)	Extraction date	Injection date	Recovery (*) (%)	
Mosquito net	1.98	April 2008	April 2008	88	
	1.98	April 2008	April 2008	93	
	1.98	April 2008	April 2008	94	
	1.91	April 2008	April 2008	99	
	1.91	April 2008	April 2008	98	
	Mean				94
	Standard deviation				4
	Relative standard deviation				4.7 %
	Confidence interval (**)				94 ± 6
	9.89	April 2008	April 2008	98	
	9.86	April 2008	April 2008	99	
	9.89	April 2008	April 2008	98	
	9.56	April 2008	April 2008	99	
	9.51	April 2008	April 2008	97	
	Mean				98
	Standard deviation				1
	Relative standard deviation				0.9 %
	Confidence interval (**)				98 ± 1
	19.7	April 2008	April 2008	99	
	19.8	April 2008	April 2008	98	
	19.7	April 2008	April 2008	98	
	19.1	April 2008	April 2008	97	
	19.8	April 2008	April 2008	97	
	Mean				98
	Standard deviation				1
	Relative standard deviation				0.9 %
	Confidence interval (**)				98 ± 1
	Mean				97
Standard deviation				3	
Relative standard deviation				3.1 %	
Confidence interval (**)				97 ± 2	

(*) Each result is the mean of 2 chromatographic injections.

(**) Student t-test with a probability of 95 %.

The average recovery for piperonyl butoxide on mosquito net for each fortification level is in the range 90-110 % with a RSD < 5 %.

1.5 Estimated limit of quantification (LOQ)

The limit of quantification (LOQ) was appraised as the concentration of deltamethrin and piperonyl butoxide yielding a peak signal of at least 5 times the baseline noise.

Active substance expressed as	LOQ (g/kg)
deltamethrin	0.01
piperonyl butoxide	0.1

2. Performance verification of the analytical method

2.1 Accuracy and reproducibility (recoveries)

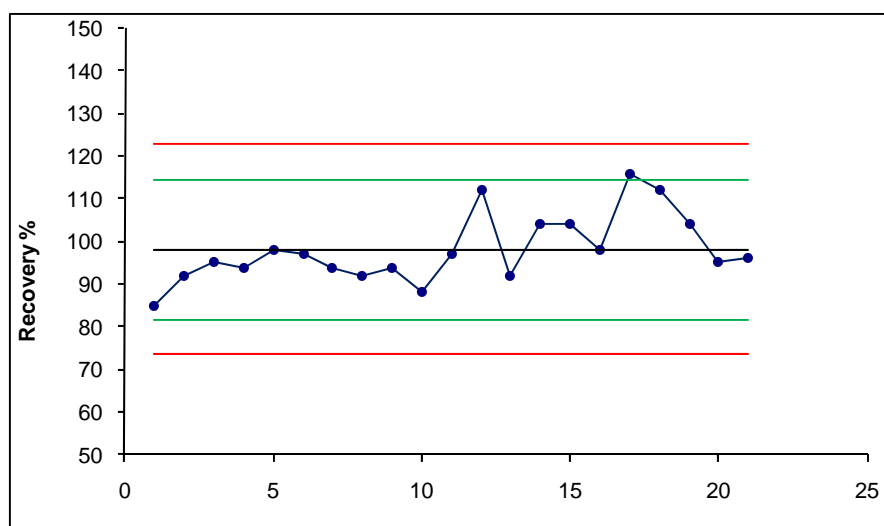
Between April 2008 and March 2009, the performance of the analytical method was controlled in the framework of several (WHOPES) Phase I and II studies by performing recoveries concurrently with the analysis of more than 830 samples of PermaNet® 3.0, 2.5 and 2.0. Recovery values were calculated by fortifying untreated net samples with known amounts of deltamethrin and piperonyl butoxide. The untreated net samples were analysed prior to spiking and showed the absence of compound interfering with determination of deltamethrin and piperonyl butoxide (< 30 % LOQ).

Recoveries for deltamethrin at 0.4 g/kg

N = 20

Mean recovery = 98.2%

RSD = 8.3%

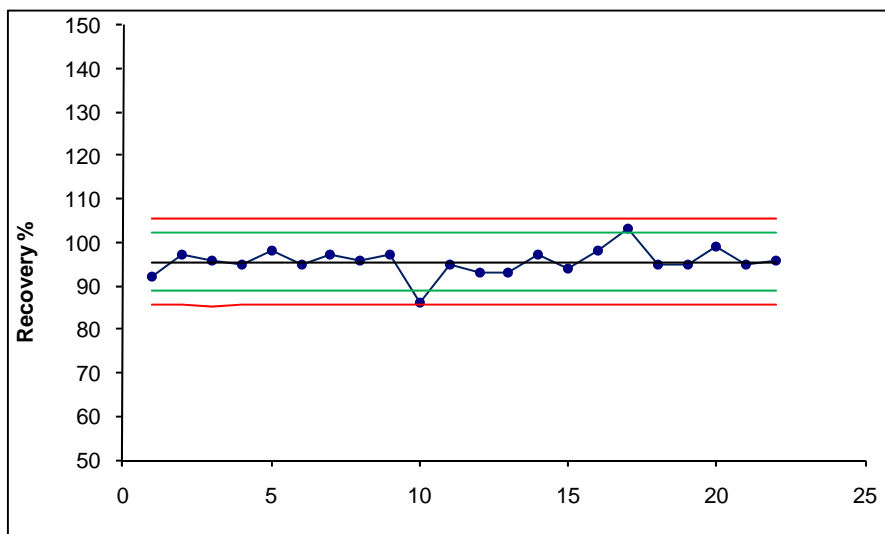


Recoveries for deltamethrin at 2 g/kg

N = 20

Mean recovery = 95.6%

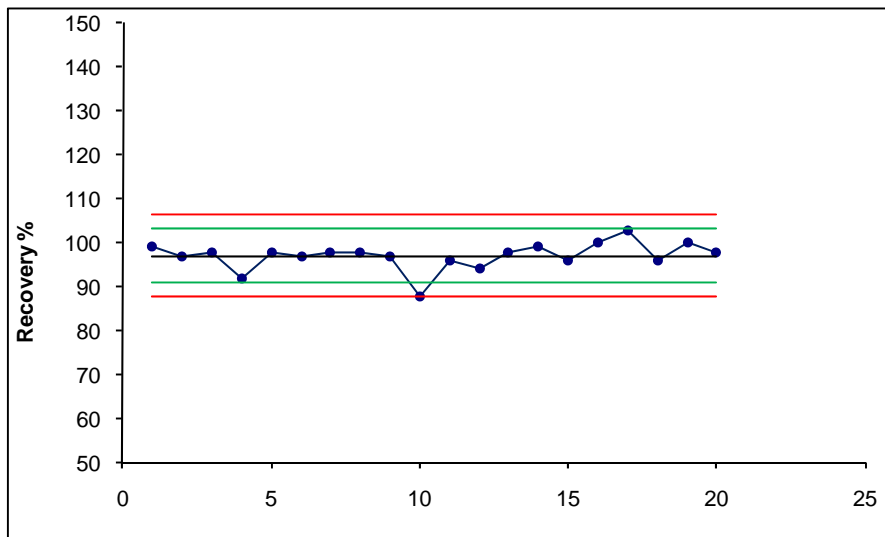
RSD = 3.5%

**Recoveries for deltamethrin at 4 g/kg**

N = 20

Mean recovery = 97.1%

RSD = 3.2%

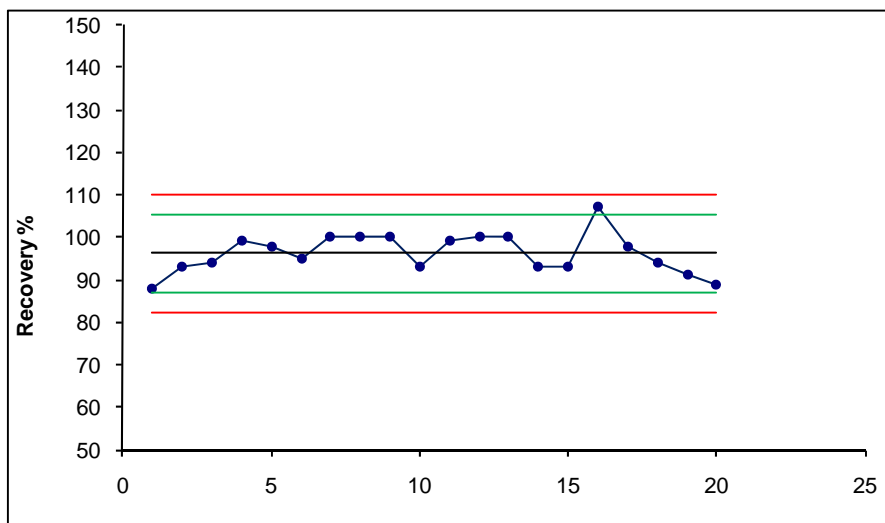


Recoveries for piperonyl butoxide at 2 g/kg

N = 20

Mean recovery = 96.2%

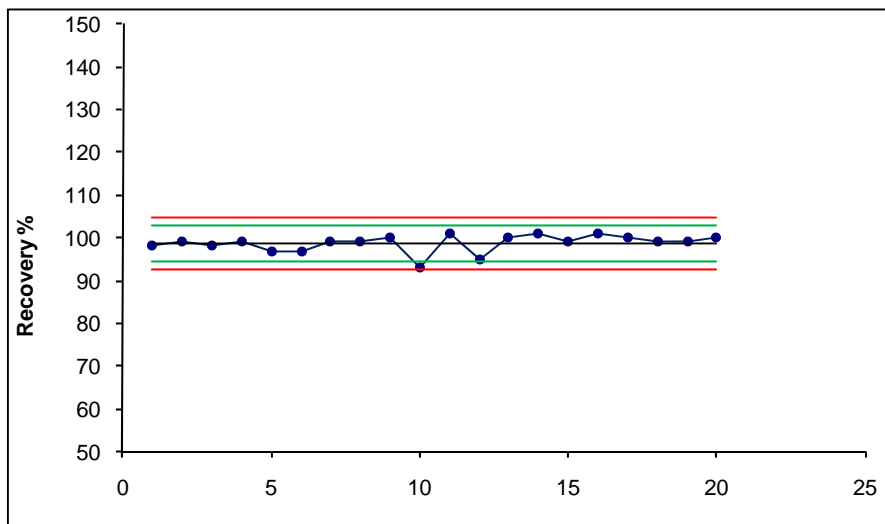
RSD = 4.8%

**Recoveries for piperonyl butoxide at 10 g/kg**

N = 20

Mean recovery = 98.7%

RSD = 2.0%

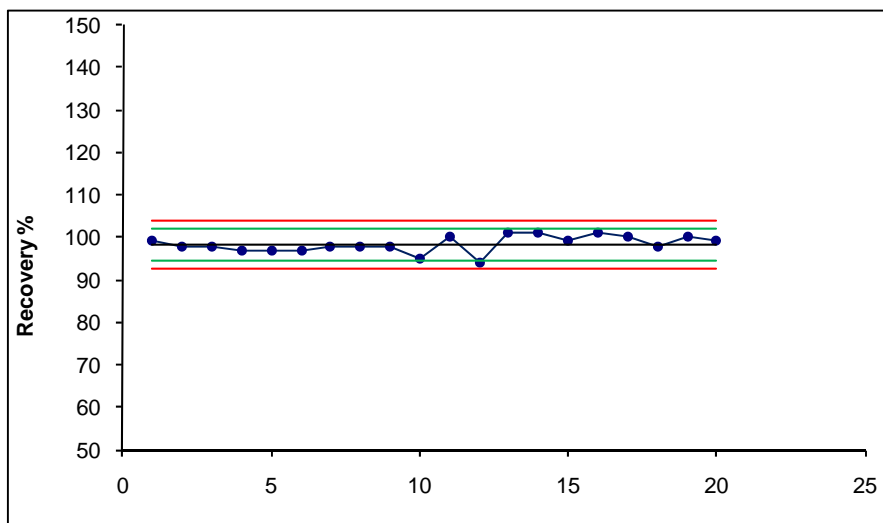


Recoveries for piperonyl butoxide at 20 g/kg

N = 20

Mean recovery = 98.4%

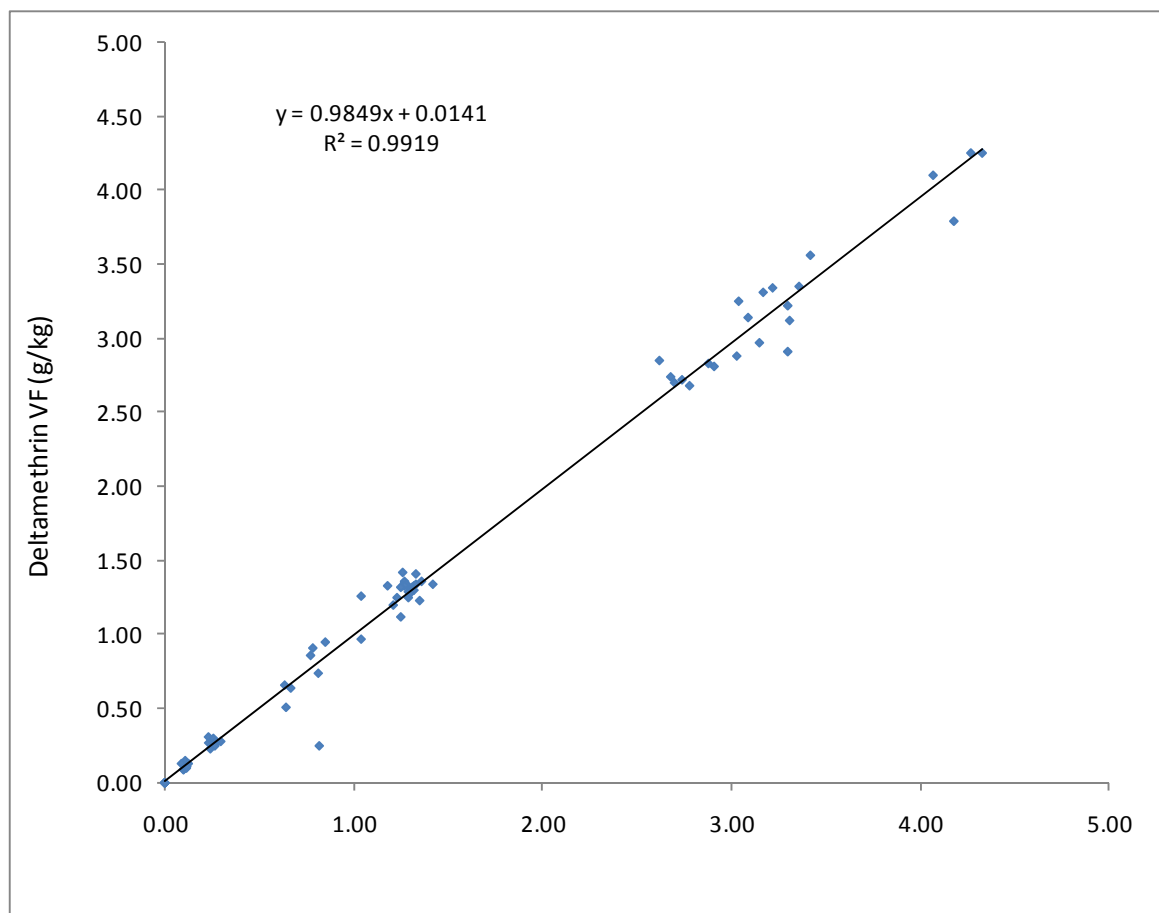
RSD = 1.9%



The control of the performance of the analytical method performed during analysis of more than 830 samples show mean recoveries of 95-99 % with RSD ranging from 1.9% to 8.3% depending on the concentration level. Very few recoveries are outside the warning limit (green line) and all the recoveries are within the action limit of the quality control chart. Recoveries performed concurrently with the analysis of samples show therefore a good accuracy and within laboratory reproducibility of the analytical method.

2.2 Comparison with another laboratory

The analytical method from CRA-W was compared with the analytical method from Vestergaard Frandsen for deltamethrin content in 90 samples of PermaNet 3.0 and 2.0. The analytical method from Vestergaard Frandsen involved extraction by heating under reflux with toluene, solvent exchange from toluene to mixture isooctane / 1,4 dioxane and chromatographic determination by High Performance Liquid Chromatography with UV Detection (HPLC-UV). The following graphs show comparable results ($R^2 > 0.99$).



3. Conclusion

The analytical method was successfully validated. The control of the performance of the analytical method performed concurrently with the analysis of more than 830 samples showed also a good accuracy and a good within laboratory reproducibility. The proposed method is considered appropriate for the determination of deltamethrin and/or piperonyl butoxide in Long-Lasting (incorporated into polyethylene) Insecticidal Mosquito Nets.

Acknowledgements

The author wish to gratefully thank the WHO Pesticides Evaluation Scheme, the LN manufacturers Vestergaard Frandsen and Intelligent Insect Control, the IRD/CREC, the London School of Tropical Hygiene & Medicine, The Institute of Tropical Medicine and the personnel (LN Team) of the Pesticides Research Department of the Walloon Agricultural Research Centre (CRA-W).