Bifenthrin + pyriproxyfen + piperonyl butoxide

415 + 715 + 33

in Technical material (TC)
and Long-Lasting Insecticide-treated Net (LN or ITN)

CIPAC 5299/m

Small scale collaborative trial

GC-FID method

ANALYTICAL METHOD FOR
LONG-LASTING INSECTICIDE-TREATED NETS CONTAINING
BIFENTHRIN, PYRIPROXYFEN AND PIPERONYL BUTOXIDE

SCOPE

This method is intended for determining bifenthrin, pyriproxyfen and piperonyl butoxide content in insecticide-treated net (ITN), incorporated into filament.

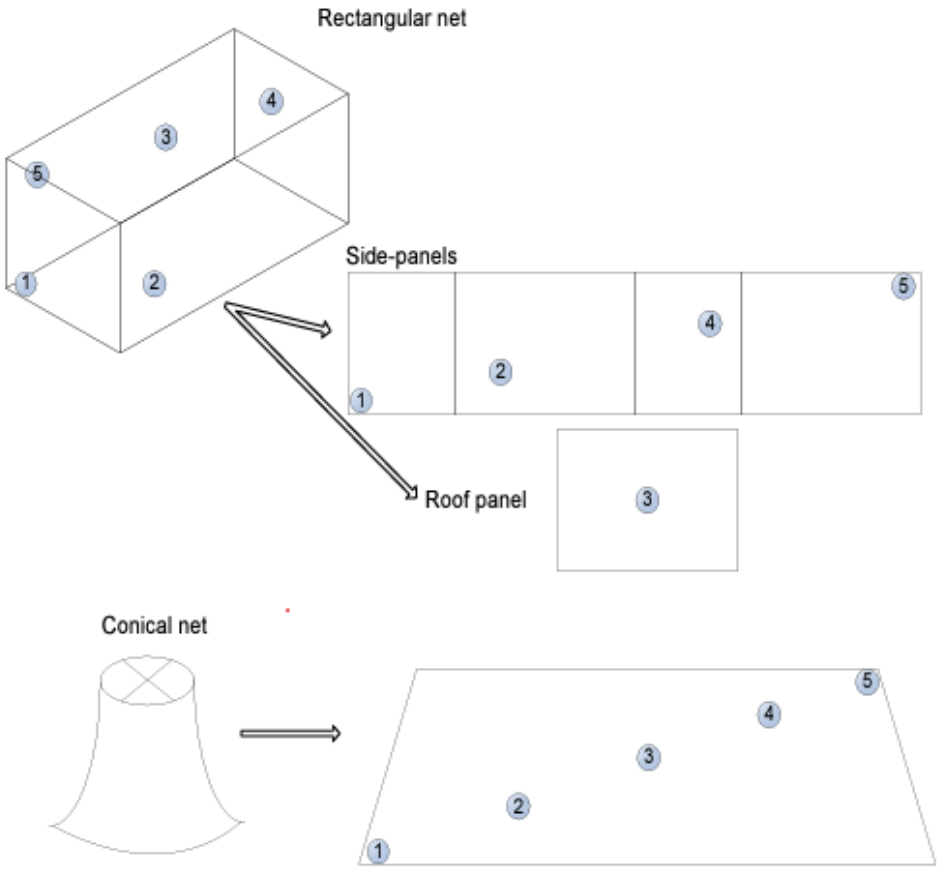
Determination of bifenthrin, pyriproxyfen and piperonyl butoxide in Technical material (TC) can be also performed by this method.

1. Sampling for ITN

This sampling procedure is suitable for net samples taken from either new or used LN/ITN.

Samples of at least 25 x 25 cm from LN/ITN are taken following the sampling method described in the specification template for insecticide treated nets or netting (LN/ITN) of the Manual on the development and use of FAO and WHO specifications for chemical pesticides, second edition, Rome and Geneva, 2022.

Fig. 1: General method for sampling rectangular and conical nets



In total 5 net pieces are taken.

The net pieces are cut in small pieces (max. 5 x 5 mm) and mixed. The net pieces can be pooled together before analytical determination or analyzed individually.

When the small pieces are pooled, they have to be carefully mixed to get a homogenous aggregated sample. The analysis of this one gives only information about the average content of active ingredient(s) in net. However, the analysis of each net pieces allows getting information about the spatial distribution of the active ingredient(s) besides the mean of content of active ingredient(s) in net.

2. Identity test

Bifenthrin	**GC.**	Use the GC method below. The retention time of bifenthrin in the sample solution should not deviate by more than 3 % from that of the calibration solution.
Pyriproxyfen	**GC.**	Use the GC method below. The retention time of pyriproxyfen in the sample solution should not deviate by more than 3 % from that of the calibration solution.
Piperonyl butoxide	**GC.**	Use the GC method below. The retention time of piperonyl butoxide in the sample solution should not deviate by more than 3 % from that of the calibration solution.

3. Bifenthrin, pyriproxyfen and piperonyl butoxide content

OUTLINE OF METHOD

The sample is extracted by heating and sonication with heptane using dicyclohexyl phthalate as internal standard. Bifenthrin, pyriproxyfen and piperonyl butoxide contents are determined by gas chromatography with flame ionisation detection (GC-FID).

REAGENTS

- Bifenthrin (BIF), certified analytical standard of known purity
- Pyriproxyfen (PYR), certified analytical standard of known purity
- Piperonyl butoxide (PBO), certified analytical standard of known purity
- Dicyclohexyl phthalate, internal standard (ISTD) of known purity
- n-Heptane, analytical reagent grade

Internal standard stock solution

Weigh, accurately to the nearest 0.1 mg, enough dicyclohexyl phthalate into a suitable volumetric flask to obtain a concentration of about 2.5 mg/mL. Add heptane and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane (solution C_{ISTD}). Mix thoroughly.

Ensure sufficient quantity of this solution is prepared for all the samples and calibration solutions to be analyzed.

Bifenthrin, pyriproxyfen and piperonyl butoxide calibration stock solutions

Weigh in duplicate, accurately to the nearest 0.1 mg, about 25 mg of bifenthrin (s_{BIF} mg), about 25 mg of pyriproxyfen (s_{PYR} mg) and about 25 mg of piperonyl butoxide (s_{PBO} mg) analytical standards into two 25 mL volumetric flasks, each flask containing the three analytical standards. Add heptane and place the flasks in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane (solutions $C_{BIF+PYR+PBO}$ and $C^*_{BIF+PYR+PBO}$). Mix thoroughly.

Bifenthrin, pyriproxyfen and piperonyl butoxide calibration working solutions

Prepare the following calibration solutions into conical flasks at room temperature, using the calibration stock solution $C_{BIF+PYR+PBO}$ as described in the below table (= calibration solutions C_1, C_2, C_3, C_4 and C_5).

Internal standard (C_{ISTD}) and bifenthrin + pyriproxyfen + piperonyl butoxide ($C_{BIF+PYR+PBO}$) solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Code	IS	Volume of BIF +PYR + PBO transferred	Bifenthrin (µg/mL), approx.	Pyriproxyfen (µg/mL), approx.	Piperonyl butoxide (µg/mL), approx	Heptane	Final volume ($V_{working\ cal\ BIF+PYR+PBO}$)
C_1	1 ml	0.3 mL	12	12	12	Up to volume	25 ml
C_2	1 ml	2 mL	80	80	80	Up to volume	25 ml
C_3	1 ml	3.5 mL	140	140	140	Up to volume	25 ml
C_4	1 ml	4 mL	160	160	160	Up to volume	25 ml
C_5	1 ml	5 mL	200	200	200	Up to volume	25 ml

$C^*_{BIF+PYR+PBO}$ is used to control the weighing of $C_{BIF+PYR+PBO}$: prepare a C^*_3 using the calibration stock solution $C^*_{BIF+PYR+PBO}$ as described in the below table (= calibration solution C^*_3).

Internal standard (C_{ISTD}) and bifenthrin + pyriproxyfen + piperonyl butoxide ($C^*_{BIF+PYR+PBO}$) solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Code	IS	Volume of BIF +PYR + PBO transferred	Bifenthrin (µg/mL), approx.	Pyriproxyfen (µg/mL), approx.	Piperonyl butoxide (µg/mL), approx	Heptane	Final volume ($V_{working\ cal\ BIF+PYR+PBO}$)
C^*_3	1 ml	3.5 mL	140	140	140	Up to volume	25 ml

APPARATUS

Capillary column fused silica, coated with (50 % trifluoropropyl)-methylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness or equivalent column with same selectivity.

Gas chromatograph capable to operate with temperature rate, equipped with flame ionisation detector (FID), split / splitless injection and automatic sampler.

Electronic integrator or data system.

Semi–micro-analytical balance : capable of ± 0.1 mg readability.

Volumetric flasks of 25 mL and of suitable volume (to prepare the internal standard stock solution).

Volumetric pipettes of 0.3 mL, 1 mL, 2 mL , 3.5 mL, 4 mL and 5 mL or electronic pipette able to dispense these volumes.

Conical flasks of 50 mL (or of suitable volume).

100 mL cap glass bottles or flasks.

Heating ultrasonic bath, capable of heating up to 80°C.

Thermostatic bath.

*Solvent filtration uni*t with 0.45 µm PTFE filters.

PROCEDURE

(a) Operating chromatographic conditions (typical)

Column: capillary column (50 % trifluoropropyl)-methylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness or equivalent material with same selectivity.

Injection system:

Injector: Split injection
Injector temperature: 240°C
Split ratio: 10 : 1
Injection volume: 1 µL

Detector system

Detector type:	Flame Ionization Detection (FID)
Detector temperature:	300°C
Oven temperature :	205 °C for 22 minutes
Gas and flow:	
Carrier :	helium, 1.5 mL/min in constant flow (with an EPC)
Makeup:	helium or nitrogen, 30 mL/min
FID:	hydrogen, 30 mL/min air (clean) : 300 mL/min
Retention times :	piperonyl butoxide *:* ± 6.5 minutes bifenthrin : ± 8.8 minutes pyriproxyfen : ± 9.1 minutes dicyclohexyl phthalate (internal standard) : ± 14.6 minutes

(b) System equilibration

Inject 1 µL of a calibration working solution C_3 before analysis until the response factor (($f_{i\ BIF}$, $f_{i\ PYR}$ and $f_{i\ PBO}$) obtained for two consecutive injections differs by less than 1.0 %.

Then inject 1 µL of the other calibration working solution C^*_3. For each active ingredient, the response factors, ($f_{i\ BIF}$ *vs* $f^*_{i\ BIF}$, $f_{i\ PYR}$ *vs* $f^*_{i\ PYR}$ and $f_{i\ PBO}$ *vs* $f^*_{i\ PBO}$) should not deviate by more than 1.0% from that of solution C_3, for each active ingredient. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula :

$$f_{i\ BIF\ or\ PYR\ or\ PBO} = \frac{I_r \times s_{BIF\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ PYR\ or\ PBO} \times V_{BIF+PYR+PBO\ transferred}}{H_{s\ BIF\ or\ PYR\ or\ PBO} \times V_{stock\ BIF+PYR+PBO} \times V_{working\ cal\ BIF+PYR+PBO}}$$

Where :

$f_{i\ BIF\ or\ PYR\ or\ PBO}$ = individual response factor, for bifenthrin, pyriproxyfen or piperonyl butoxide

$H_{s\ BIF\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, pyriproxyfen or piperonyl butoxide in the calibration solution (C_3 or C^*_3)

I_r = peak area of internal standard in the calibration solution (C_3 or C^*_3)

$S_{BIF\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+PYR+PBO}$ and $C^*_{BIF+PYR+PBO}$, in mg

$P_{BIF\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+PYR+PBO}$ and $C^*_{BIF+PYR+PBO}$, in g/kg

$V_{BIF+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+PYR+PBO}$ or $C^*_{BIF+PYR+PBO}$) transferred to prepare the working calibration solution (C_3 or C^*_3), in mL (= 3.5 mL)

$V_{stock\ BIF+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+PYR+PBO}$ or $C^*_{BIF+PYR+PBO}$), in mL (= 25 mL)

$V_{working\ cal\ BIF+PYR+PBO}$ = total volume of the calibration working solution (C_3 or C^*_3), in mL (= 25 mL)

If the peak retention times differ significantly from the values given, then adjust the flow rate accordingly.

(c) Preparation of samples solutions for bifenthrin TC

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add heptane and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane. Mix thoroughly.

Transfer precisely by pipette 3.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 20.5 mL of heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Blank solution should be prepared following the previously described conditions, but without adding any TC sample (= Solution “blank ISTD”).

(d) Preparation of samples solutions for pyriproxyfen TC

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add heptane and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane. Mix thoroughly.

Transfer precisely by pipette 3.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 20.5 mL of heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Blank solution should be prepared following the previously described conditions, but without adding any TC sample (= Solution “blank ISTD”).

(e) Preparation of samples solutions for piperonyl butoxide TC

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add heptane and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane. Mix thoroughly.

Transfer precisely by pipette 3.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 20.5 mL of heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Blank solution should be prepared following the previously described conditions, but without adding any TC sample (= Solution "blank ISTD").

(f) Preparation of samples solutions for ITN

Weigh, accurately to the nearest 0.1 mg, about 500 mg of ITN sample cut in small pieces into a 100 mL cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and 24 mL of heptane. Put the flask in a heating ultrasonic bath at 80 °C for 60 minutes. Note that the net sample is not dissolved. Allow the solution to cool to room temperature and mix thoroughly. Filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Blank solution should be prepared following the previously described conditions, but without adding any LN/ITN sample (= Solution "blank ISTD").

(g) Determination.

Inject blank solutions and calibration working solutions (C_3 and C^*_3) first. The calibration working solution C^*_3 is used to check the accuracy of the weighing of the calibration solution $C_{BIF+PYR+PBO}$. The following sequence is advised: solvent, blank ISTD, C_3 in duplicate and C^*_3 in duplicate. Then, inject the sample extracts in duplicate. Each 2 to 4 sample extracts are bracketed with a calibration solution (C_1 to C_5), as follows: calibration solution C_1, sample solution $S1_A$, sample solution $S1_B$, sample solution $S2_A$, sample solution $S2_B$, calibration solution C_2, sample solution $S3_A$, sample solution $S3_B$, sample solution $S4_A$, sample solution $S4_B$, calibration solution C_3 and so on for further samples. Measure the relevant peak areas.

(h) Calculation.

Quantitative determination of bifenthrin, pyriproxyfen and piperonyl butoxide in the sample solutions is carried out by comparing the ratio of peaks area of bifenthrin,

pyriproxyfen or piperonyl butoxide to the peak area of dicyclohexyl phthalate in the sample solutions with that of the standard solutions, on basis of a calibration curve calculated with standard solutions (C_1 to C_5) bracketing the sample solutions.

The calibration curves for bifenthrin, pyriproxyfen and piperonyl butoxide are obtained by the internal standard calibration method from the injection of bifenthrin, pyriproxyfen and piperonyl butoxide standard solutions containing dicyclohexyl phthalate and plotting the ratio of peaks areas (peak area BIF, PYR or CFP / peak area ITSD) versus the bifenthrin, pyriproxyfen or piperonyl butoxide concentration (in µg/mL). Calculate the equation of the linear regression obtained.

- y- axis = $\frac{H_{w\ BIF\ or\ PYR\ or\ PBO}}{I_q}$
- x- axis = $\frac{S_{BIF\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ PYR\ or\ PBO} \times V_{BIF+PYR+PBO\ transferred}}{V_{stock\ BIF+PYR+PBO} \times V_{working\ cal\ BIF+PYR+PBO}}$

Where :

$H_{w\ BIF\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, pyriproxyfen or piperonyl butoxide in the sample solution

I_q = peak area of internal standard in the sample solution

$S_{BIF\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+PYR+PBO}$, in mg

$P_{BIF\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+PYR+PBO}$, in g/kg

$V_{BIF+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+PYR+PBO}$) transferred to prepare the working calibration solutions (C_1 to C_5), in mL (= 0.3, 2, 3.5, 4 and 5 mL, respectively)

$V_{stock\ BIF+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+PYR+PBO}$), in mL (= 25 mL)

$V_{working\ cal\ BIF+PYR+PBO}$ = total volume of the calibration working solution (C_1 to C_5), in mL (= 25 mL)

The amount of bifenthrin, pyriproxyfen or piperonyl butoxide in the samples is expressed in g of bifenthrin, in g of pyriproxyfen and in g of piperonyl butoxide per kg of sample; taking into account of dilution factor and sample weight.

Content of bifenthrin, pyriproxyfen or piperonyl butoxide in the samples :

$$= \frac{C_{BIF\ or\ PYR\ or\ PBO} \times D}{W}\ g/kg$$

Where :

$C_{BIF\ or\ PYR\ or\ PBO}$	=	concentration of bifenthrin, pyriproxyfen or piperonyl butoxide in the sample solution, in µg/mL, found using the equation of the calibration curve
D	=	dilution factor of the sample solution (= 25 for ITN, = 25*25/3.5 for TC)
W	=	weight of the sample, in g

Bifenthrin:

Repeatability r = g/kg at g/kg active ingredient content (ITN/LN)

Reproducibility R = g/kg at g/kg active ingredient content (ITN/LN)

Pyriproxyfen:

Repeatability r = g/kg at g/kg active ingredient content (ITN/LN)

Reproducibility R = g/kg at g/kg active ingredient content (ITN/LN)

Piperonyl butoxide:

Repeatability r = g/kg at g/kg active ingredient content (ITN/LN)

Reproducibility R = g/kg at g/kg active ingredient content (ITN/LN)

4. Data handling

4.1 Response factor of the calibration solutions

After equilibration of the chromatographic system, inject the 2 calibration working solutions C_3 and C^*_3 before analysis to ensure that the relative response factors for C^*_3 ($f_{i\ BIF}$ *vs* $f^*_{i\ BIF}$, $f_{i\ PYR}$ *vs* $f^*_{i\ PYR}$ and $f_{i\ PBO}$ *vs* $f^*_{i\ PBO}$) does not deviate by more than 2.0 % from that of solution C_3, for each active ingredient. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula :

$$f_{i\ BIF\ or\ PYR\ or\ PBO} = \frac{I_r \times s_{BIF\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ PYR\ or\ PBO} \times V_{BIF+PYR+PBO\ transferred}}{H_{s\ BIF\ or\ PYR\ or\ PBO} \times V_{stock\ BIF+PYR+PBO} \times V_{working\ cal\ BIF+PYR+PBO}}$$

Where :

$f_{i\ BIF\ or\ PYR\ or\ PBO}$ = individual response factor, for bifenthrin, pyriproxyfen or piperonyl butoxide

$H_{s\ BIF\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, pyriproxyfen or piperonyl butoxide in the calibration solution (C_3 or C^*_3)

I_r = peak area of internal standard in the calibration solution (C_3 or C^*_3)

$S_{BIF\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+PYR+PBO}$ and $C^*_{BIF+PYR+PBO}$, in mg

$P_{BIF\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+PYR+PBO}$ and $C^*_{BIF+PYR+PBO}$, in g/kg

$V_{BIF+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+PYR+PBO}$ or $C^*_{BIF+PYR+PBO}$) transferred to prepare the working calibration solution (C_3 or C^*_3), in mL (= 3.5 mL)

$V_{stock\ BIF+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+PYR+PBO}$ or $C^*_{BIF+PYR+PBO}$), in mL (= 25 mL)

$V_{working\ cal\ BIF+PYR+PBO}$ = total volume of the calibration working solution (C_3 or C^*_3), in mL (= 25 mL)

4.2 Determination of bifenthrin, pyriproxyfen or piperonyl butoxide in the sample solutions

Quantitative determination of bifenthrin, pyriproxyfen and piperonyl butoxide in the sample solutions is carried out by comparing the ratio of peaks area of bifenthrin, pyriproxyfen or piperonyl butoxide to the peak area of dicyclohexyl phthalate in the

sample solutions with that of the standard solutions, on basis of a calibration curve calculated with standard solutions (C_1 to C_5) bracketing the sample solutions.

The calibration curves for bifenthrin, pyriproxyfen and piperonyl butoxide are obtained by the internal standard calibration method from the injection of bifenthrin, pyriproxyfen and piperonyl butoxide standard solutions containing dicyclohexyl phthalate and plotting the ratio of peaks areas (peak area BIF, PYR or CFP / peak area ITSD) versus the bifenthrin, pyriproxyfen or piperonyl butoxide concentration (in µg/mL). Calculate the equation of the linear regression obtained.

- y- axis = $\frac{H_{w\ BIF\ or\ PYR\ or\ PBO}}{I_q}$
- x- axis = $\frac{S_{BIF\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ PYR\ or\ PBO} \times V_{BIF+PYR+PBO\ transferred}}{V_{stock\ BIF+PYR+PBO} \times V_{working\ cal\ BIF+PYR+PBO}}$

Where :

$H_{w\ BIF\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, pyriproxyfen or piperonyl butoxide in the sample solution

I_q = peak area of internal standard in the sample solution

$S_{BIF\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+PYR+PBO}$, in mg

$P_{BIF\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+PYR+PBO}$, in g/kg

$V_{BIF+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+PYR+PBO}$) transferred to prepare the working calibration solutions (C_1 to C_5), in mL (= 0.3, 2, 3.5, 4 and 5 mL, respectively)

$V_{stock\ BIF+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+PYR+PBO}$), in mL (= 25 mL)

$V_{working\ cal\ BIF+PYR+PBO}$ = total volume of the calibration working solution (C_1 to C_5), in mL (= 25 mL)

The amount of bifenthrin, pyriproxyfen or piperonyl butoxide in the samples is expressed in g of bifenthrin, in g of pyriproxyfen and in g of piperonyl butoxide per kg of sample; taking into account of dilution factor and sample weight.

Content of bifenthrin, pyriproxyfen or piperonyl butoxide in the samples :

$$= \frac{C_{BIF\ or\ PYR\ or\ PBO} \times D}{1000 \times W}\ g/kg$$

Where :

$C_{BIF\ or\ PYR\ or\ PBO}$ = concentration of bifenthrin, pyriproxyfen or piperonyl butoxide in the sample solution, in µg/mL, found using the equation of the calibration curve

D = dilution factor of the sample solution (= 25 for ITN,
= 25*25/3.5 for TC)

W = weight of the sample, in g