Centre wallon de Recherches agronomiques (CRA-W)
Département Connaissance et Valorisation des Produits (D4)
Unité Produits de protection, de contrôle et résidus (U10)
Bâtiment Rachel Carson
Rue du Bordia, 11
B-5030 GEMBLOUX
BELGIQUE
Tél. : +32 (0) 81 87 40 06
Fax : +32 (0) 81 87 40 16
www.cra.wallonie.be

Deltamethrin + Broflanilide

333 + 994

in Long-Lasting Insecticidal Net (LN),
coated onto polyester

Small scale collaborative trial

HPLC-DAD method

5411/m

DELTAMETHRIN and BROFLANILIDE

333 and 994

Deltamethrin, see CIPAC L, *p* 45.
Broflanilide, see CIPAC P, *p* 21.

DELTAMETHRIN and BROFLANILIDE LONG LASTING INSECTICIDAL NETS

333/LN/M../- and **994**/LN/M../-

SCOPE

This method is intended for determining deltamethrin and broflanilide content in long-lasting insecticidal net (LN), coated onto polyester.

1 Sampling. This sampling procedure is suitable for net samples taken from either new or used LN. Samples of at least 25 x 25 cm from LN are taken following the sampling method described in the specification template for long-lasting insecticidal nets or netting (LN) of the Manual on the development and use of FAO and WHO specifications for chemical pesticides, second edition[1], Rome and Geneva, 2022.

[1] FAO and WHO. 2022. Manual on the development and use of FAO and WHO specifications for chemical pesticides – Second edition. Rome and Geneva. https://doi.org/10.4060/cb8401en.

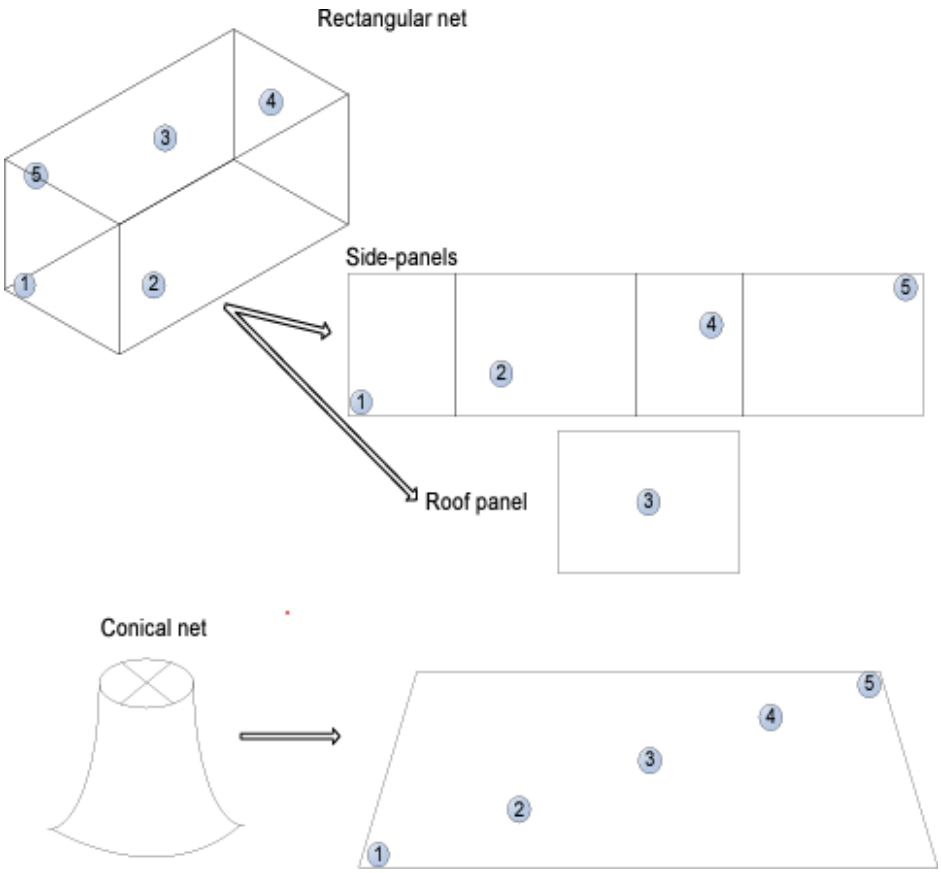

Fig. 1 General method for sampling rectangular and conical nets

Take a total of 5 net pieces.

Cut the net pieces into small pieces (max. 5 x 5 mm) and mix. The net pieces can be pooled together before analytical determination or analysed individually.

When the small pieces are pooled, mix them carefully to get a homogenous aggregated sample. The analysis of the pooled sample gives only information about the average content of active ingredient(s) in net. However, the analysis of each net pieces gives information about the spatial distribution of the active ingredient(s) besides the mean of content of active ingredient(s) in net.

2 Identity tests

2.1 Deltamethrin: HPLC. Use the HPLC method below. The retention time of deltamethrin in the sample solution should not deviate by more than 1% from that of the calibration solution.

2.2 Broflanilide: HPLC. Use the HPLC method below. The retention time of broflanilide in the sample solution should not deviate by more than 1% from that of the calibration solution.

3 Deltamethrin and broflanilide

OUTLINE OF METHOD

The sample is extracted with acetonitrile using dibutyl phthalate as internal standard. Deltamethrin and broflanilide contents are determined by reverse phase liquid chromatography with ultraviolet detection (HPLC-DAD).

REAGENTS

Deltamethrin (DM), certified analytical standard of known purity

Broflanilide (BFA), certified analytical standard of known purity

Dibutyl phthalate, internal standard (ISTD) of known purity

Acetonitrile, analytical reagent and HPLC grade

Methanol, HPLC grade

Water, HPLC grade

Mobile phase. methanol/water 72:28, v/v

Internal standard stock solution. Prepare a stock of 1.0 mg/ml internal standard solution. For example, weigh, accurately to the nearest 0.1 mg, about 100 mg of dibutyl phthalate into a 100 ml volumetric flask. Add acetonitrile 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 acetonitrile (solution $ISTD_{stock}$). Mix thoroughly.

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

Calibration stock solutions. Weigh in duplicate, accurately to the nearest 0.1 mg, about 25 mg of deltamethrin and about 45 mg of broflanilide analytical standards (s mg) into two separate 100 ml volumetric flasks, each flask containing both analytical standards. Add acetonitrile 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 acetonitrile (solutions C_{DM+BFA} and C^*_{DM+BFA}). Mix thoroughly.

Calibration working solutions. Prepare the following calibration solutions into conical flasks at room temperature, using the calibration stock solution C_{DM+BFA} as described in the below table (= calibration solutions C_1, C_2, C_3, C_4 and C_5). Add the internal standard and deltamethrin + broflanilide solutions at 20 °C ± 1 °C and using a volumetric pipette.

Code	$ISTD_{stock}$	C_{DM+BFA}	Deltamethrin (µg/ml), approx.	Broflanilide (µg/ml), approx.	Acetonitrile	Final volume
C_1	**1 ml**	**2 ml**	25	45	Up to volume	20 ml
C_2	**1 ml**	**4 ml**	50	90	Up to volume	20 ml
C_3	**1 ml**	**5 ml**	62.5	112.5	Up to volume	20 ml
C_4	**1 ml**	**6 ml**	75	135	Up to volume	20 ml
C_5	**1 ml**	**8 ml**	100	180	Up to volume	20 ml

Use C^*_{DM+BFA} to check the accuracy of the weighing of C_{DM+BFA}: for that, prepare a C^*_3 using the calibration stock solution C^*_{DM+BFA} as described in the below table (= calibration solution C^*_3).

Add the internal standard and deltamethrin + broflanilide solutions at 20 °C ± 1 °C and using a volumetric pipette.

Code	$ISTD_{stock}$	C^*_{DM+BFA}	Deltamethrin (µg/ml), approx.	Broflanilide (µg/ml), approx.	Acetonitrile	Final volume
C^*_3	**1 ml**	**5 ml**	62.5	112.5	Up to volume	20 ml

Store the stock and working calibration solutions out of direct sunlight and in a refrigerated (<10 °C) zone.

APPARATUS

High performance liquid chromatograph (HPLC), equipped with a constant flow pump, an auto-sampler capable of delivering 10 µl, a column oven and an UV detector capable of measuring at 236 nm.

Electronic integrator or data system

HPLC column, stainless steel, 250 mm x 4.6 mm (i.d.), packed with BDS Hypersil™ C18 (5 µm), or equivalent material with same selectivity (Note 1)

PTFE filter, with maximum 0.45 µm pore size

Ultrasonic bath

PROCEDURE

(a) Liquid chromatographic conditions (typical)

Column temperature	30 °C	
Flow rate	isocratic, 1.25 ml/min	
Injection volume	10 µl	
Detector wavelength	236 nm	
Run time	about 50 min. Run time may be increased for column clean-up to avoid interferences of co-formulants.	
Retention times	broflanilide:	about 10 min
	dibutyl phthalate:	about 11 min
	deltamethrin:	about 41 min

(b) System equilibration. Pump sufficient mobile phase through the column to equilibrate the system. Inject 10 µl portions of the 2 calibrations working solutions C_3 and C^*_3 before analysis and repeat the injections until retention times does not deviate by more than 1.0% from the mean for three successive injections, for both active ingredients. Ensure that and relative response factors ($f_{i\,DM}$ *vs* $f^*_{i\,DM}$ and $f_{i\,BFA}$ *vs* $f^*_{i\,BFA}$) does not deviate by more than 2.0%, for both active ingredients. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula:

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

where:

$f_{i\ DM\ or\ BFA}$ = individual response factor, for deltamethrin or broflanilide

$H_{s\ DM\ or\ BFA}$ = peak area of deltamethrin or broflanilide 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)

$s_{DM\ or\ BFA}$ = mass of deltamethrin or broflanilide reference standard in the calibration stock solution C_{DM+BFA} and C^*_{DM+BFA} (mg)

$P_{DM\ or\ BFA}$ = purity of deltamethrin or broflanilide reference standard used to prepare the calibration stock solution C_{DM+BFA} and C^*_{DM+BFA} (g/kg)

$V_{DM+BFA\ transferred}$ = volume of the calibration stock solution (C_{DM+BFA} or C^*_{DM+BFA}) transferred to prepare the working calibration solution (C_3 or C^*_3), (ml, typically 5 ml)

$V_{stock\ DM+BFA}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+BFA} or C^*_{DM+BFA}) (ml, typically 100 ml)

$V_{working\ cal\ DM+BFA}$ = total volume of the calibration working solution (C3 or C*3), (ml, typically 20 ml)

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

(c) Preparation of samples solutions for LN. Weigh in duplicate, accurately to the nearest 0.1 mg, about 500 mg of LN sample cut in small pieces into a 50 ml extraction glass bottle with a cap. Add precisely at 20 °C ± 1 °C and by volumetric pipette, 1 ml of internal standard stock solution and 19 ml of acetonitrile. Tighten the bottle cap, gently shake the bottle to ensure that all the net sample is immersed in the extraction solvent. Put the sample bottle into an ultrasonic bath and sonicate for 15 min. 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 PTFE filter with maximum 0.45 µm pore size, before filling an injection vial (sample solutions S_1 and S_2).

Blank solution should be prepared following the previously described conditions, but without adding any LN sample (= Solution "blank ISTD"). For example, dilute 1 ml of internal standard stock solution into 19 ml of acetonitrile before filling an injection vial.

(d) Determination. Inject blank solutions and calibration working solutions (C_3 and C^*_3) first. Use the calibration working solution C^*_3 to check the accuracy of the weighing of the calibration solution C_{DM+BFA}. The following sequence is advised: solvent, blank ISTD, C_3 in duplicate and C^*_3 in duplicate. Then, inject the sample extracts in duplicate. Bracket each 2 to 4 sample extracts with a calibration solution (C_1 to C_5), as follows: calibration solution C_1, calibration solution C_1, sample solution S_1, sample solution S_1, sample solution S_2, sample solution S_2, calibration solution C_2, calibration solution C_2, and so on for further samples (C_1, C_1, S_1, S_1, S_2, S_2, C_2, C_2, S_3,…). Measure the relevant peak areas.

(e) Calculation. Calculate the content of deltamethrin and broflanilide in the sample solutions by comparing the ratio of peak area of deltamethrin or broflanilide to the peak area of dibutyl phthalate in the sample solutions with that of the standard solutions, on basis of a calibration curve established with standard solutions (C_1 to C_5) bracketing the sample solutions. Set up the calibration curves for deltamethrin and broflanilide by plotting the ratio of peaks areas (peak area of deltamethrin or broflanilide *vs* peak area of dibutyl phthalate) versus the deltamethrin or broflanilide concentration in µg/ml. Calculate the equation of the linear regression obtained.

$$\boldsymbol{y - axis} = \frac{\boldsymbol{H_{w\ DM\ or\ BFA}}}{\boldsymbol{I_q}}$$

$$\boldsymbol{x - axis} = \frac{\boldsymbol{s_{DM\ or\ BFA}} \times \boldsymbol{P_{DM\ or\ BFA}} \times \boldsymbol{V_{DM+BFA\ transferred}}}{\boldsymbol{V_{stock\ DM+BFA}} \times \boldsymbol{V_{working\ cal\ DM+BFA}}}$$

where:

$H_{w\ DM\ or\ BFA}$ = peak area of deltamethrin or broflanilide in the sample solution

I_q = peak area of internal standard in the sample solution

$s_{DM\ or\ BFA}$ = mass of deltamethrin or broflanilide reference standard in the calibration stock solution C_{DM+BFA} (mg)

$P_{DM\ or\ BFA}$ = purity of deltamethrin or broflanilide reference standard used to prepare the calibration stock solution C_{DM+BFA} (g/kg)

$V_{DM+BFA\ transferred}$ = volume of the calibration stock solution (C_{DM+BFA}) transferred to prepare the working calibration solutions (C_1 to C_5), in ml (typically 2, 4, 5, 6 and 8 ml, respectively)

$V_{stock\ DM+BFA}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+BFA}) (ml, typically 100 ml)

$V_{working\ cal}$ = total volume of the calibration working solution (C_1 to C_5) $_{DM+BFA}$ (ml, typically 20 ml)

Express the amount of deltamethrin and broflanilide in the samples in g of deltamethrin and in g of broflanilide per kg of sample; taking into account of dilution factor and sample weight.

Content of deltamethrin or broflanilide in the samples:

$$= \frac{C_{DM\ or\ BFA} \times D}{W} g/kg$$

where:

$C_{DM\ or\ BFA}$ = concentration of deltamethrin or broflanilide in the sample solution (µg/ml), found using the equation of the calibration curve

D = dilution factor of the sample solution (ml, typically 20 ml)

W = weight of the sample (mg)

Deltamethrin:
Repeatability r = xxx g/kg at xxx g/kg active ingredient content (LN)
Reproducibility R = xxx g/kg at xxx g/kg active ingredient content (LN)

Broflanilide:
Repeatability r = xxx g/kg at xxx g/kg active ingredient content (LN)
Reproducibility R = xxx g/kg at xxx g/kg active ingredient content (LN)

Note 1 The following columns were successfully used in the CIPAC full scale collaborative trial:

To be added after the full scale collaborative trial

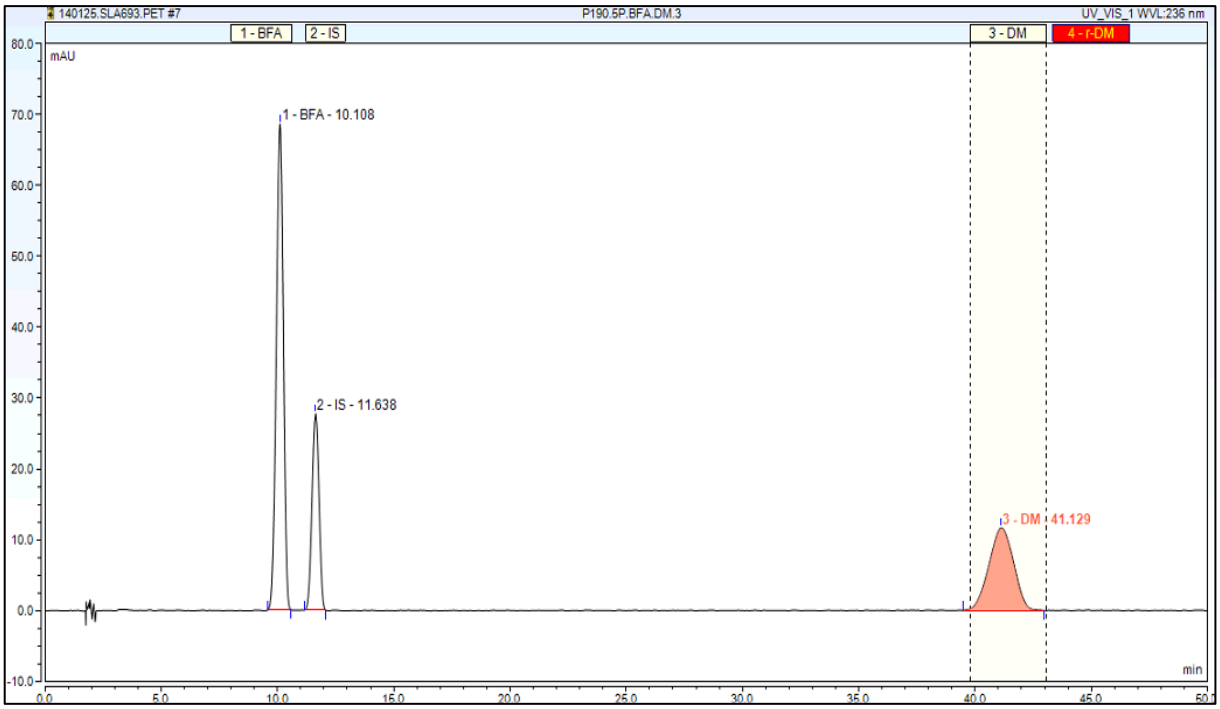

Fig. 2 Typical chromatogram of calibration solution C_3

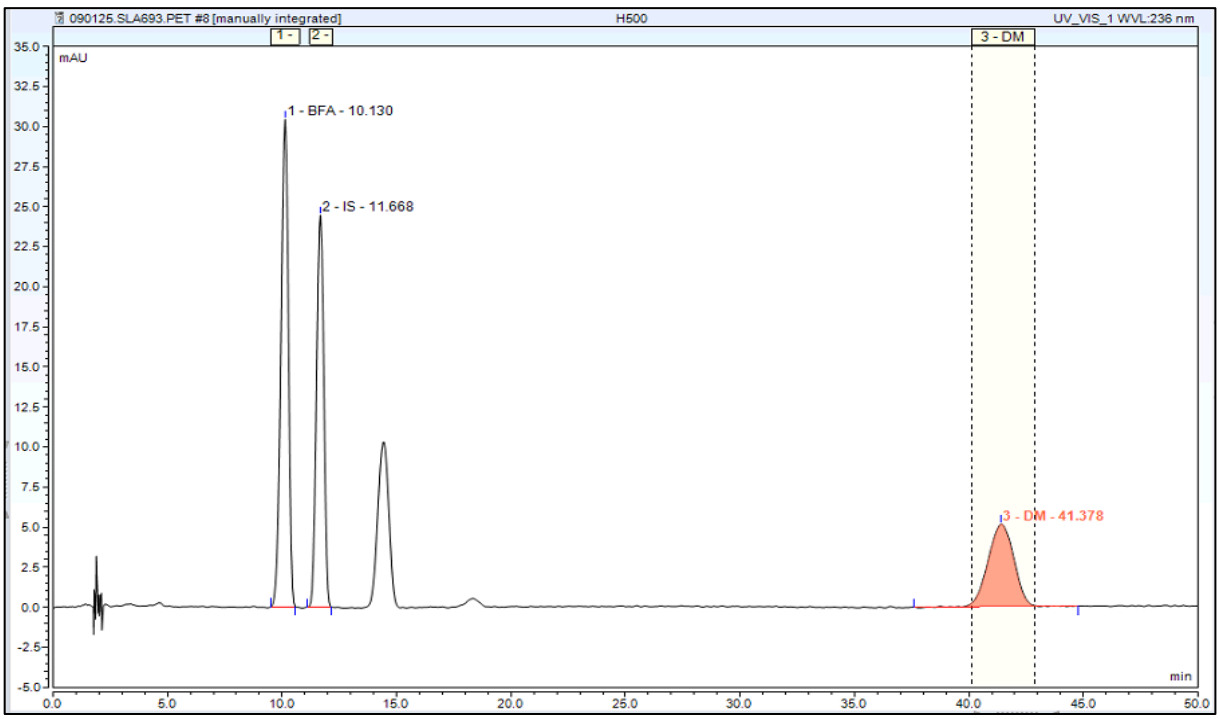

Fig. 3 Typical chromatogram of LN sample