OBSOLETE - *This method was set up and validated at a time when few multi-residue methods were available. Nowadays, more efficient and powerful multi-residue methods are being applied.*

CORESTA RECOMMENDED METHOD N° 30

DETERMINATION OF RESIDUES OF THE SUCKERCIDE FLUMETRALIN (PRIME PLUS, CGA-41065) ON TOBACCO

(June 1991)

0. FOREWORD

During the last years, the Pesticide Sub-Group of CORESTA has been testing analytical methods to quantify the residues of Flumetralin on tobacco. This exercise has been carried out conjointly with the Agronomy Group, which has organized the application of the chemical in the field and the preparation of the samples. As the results reflect all types of dispersions (field treatment, differing climates, influences during and after treatment, sampling, inter- and intra-laboratory biases), no statement about a precision of the usual type can be made. However, an estimation of the precision of the method *via* the coefficients of variation at different residue levels will be given.

Three parameters seemed to be decisive for the usefulness of the method, (1) Reliability of the results, (2) Simplicity of the analytical procedure and (3) Low-cost equipment. The majority of the Pesticide Sub-Group was in favour of a method which was adapted by Len Toet of the Tobacco Research Board, Harare, Zimbabwe, for the needs of this exercise. Valuable details were added by group members (Norman Lorton, Rothmans UK). The figures given in this report represent the results of the Joint Experiment JE-24 (samples by A. Beuchat, Deltafina, Italy).

1. SAMPLE PREPARATION

1.1. Weigh 10 g of ground tobacco and place it in a blender jar (Waring blender type), add 200 cm^3 of methanol/water (9+1) and three spatulas of celite or BDH Hyflo Super Cell. Macerate for five minutes (high speed).

1.2. Do not rinse the blender blades into the jar. Let the sample settle down.

1.3. Filter through a 540 fluted filter paper containing three spatulas of celite (another suitable method uses Buchner funnels with sintered glass, filter paper and Hyflo Super Cell).

1.4. Transfer a 40 cm^3 aliquot into a 500 cm^3 separating funnel. Add 160 cm^3 saturated, aqueous sodium chloride (NaCl) solution, 160 cm^3 distilled water (equivalent to 320 cm^3 of 15% aqueous NaCl) and 50 cm^3 methylene chloride. Shake well and release the pressure. Leave to separate. Draw the bottom phase (methylene chloride) through a funnel containing sodium sulphate (Na_2SO_4). Repeat the extraction step with 2 x 50 cm^3 methylene chloride.

1.5. Wash the funnel containing Na_2SO_4 with a further 15 cm^3 methylene chloride.

1.6. Evaporate to dryness (bath temperature 40°C)

1.7. Prepare a Florisil column (5 g filling, moisture content of Florisil 8%) and pre-wash with 50 cm^3 petrol ether (pentane).

1.8. Dissolve the residues of the evaporation step (1.6) in 10 cm^3 petrol ether and add on top of the Florisil column (using a funnel with glass wool if sample contains solid particles).

1.9. Rinse the flask twice more with 10 cm^3 petrol ether each time and add the solutions to the Florisil column.

1.10. Elute with an additional 30 cm^3 petrol ether and discard the petrol ether fractions.

1.11. Elute with 50 cm^3 of petrol ether/diethyl ether (9+1). Collect this fraction in a 100 cm^3 flask.

1.12. Evaporate to almost dryness.

1.13. Make up to 10 cm^3 with petrol ether and then make 1:10 dilutions if necessary for those samples whose concentrations are above the calibration range. The sample is ready for GC analysis. Suitable conditions see under 2.

Spiking : The recovery of this method can be checked by spiking the tobacco sample at the 1 ppm (1 µg/g) level.

2. SUITABLE GC CONDITIONS

2.1. Packed column :

Length:	1 m
Material:	11% OV17 - QF1 (1+1)
Column temperature:	225°C
Injector temperature:	235°C
Detector type:	ECD
Detector temperature:	300°C
Gas flow:	50 cm^3/min (N_2 or Ar/CH_4 95+5)

The calibration standards should be in the range of 10^{-7} g/cm^3. Injection volume 1-5 mm^3.

2.2. Capillary column :

Dimensions:	50 m x 0.32 mm i.d.
Type:	WCOT fused silica
Stationary phase:	CP-SIL-19 CB
Film thickness:	0.19 µm
Carrier gas:	Helium
Inlet pressure:	170 kPa
Injector type:	Split/splitless
Injection mode:	Split

Temperature:	250°C
Split-flow:	30 cm^3/min
Detector type:	ECD
Make-up gas:	Nitrogen
Make-up flow:	50 cm^3/min
Temperature:	300°C
Oven program:	Start at 150°C with 10°C/min to 280°C, then 11 min at 280°C

3. ASSESSMENT OF RESULTS

3.0. This assessment uses a total of 81 results which have been contributed by six laboratories.

3.1. Recovery :

The recovery can vary from 66-100% (mean of five laboratories: 84%) and should always be determined during the analysis of each batch of samples.

3.2. Blank value :

Based on 27 results of 4 laboratories, a blank value of 2.8 ppm Flumetralin on untreated leaves was determined (coefficient of variation 15,0%). These results could include a possible carry-over via the soil or from treatments of adjacent fields.

3.3. Range :

The method was tested for a range from zero (completely untreated) to about 110 ppm residue.

3.4. Overall precision of the method (see foreword) :

The coefficient of variation (standard deviation expressed as percentage of mean value) was 16.7%. No dependency on the residue level was noted.

OBSOLETE - *This method was set up and validated at a time when few multi-residue methods were available. Nowadays, more efficient and powerful multi-residue methods are being applied.*

CORESTA RECOMMENDED METHOD N° 31

DETERMINATION OF RESIDUES OF THE SUCKERCIDE PENDIMETHALIN (ACCOTAB, STOMP) ON TOBACCO

(June 1991)

0. FOREWORD

During the last years, the Pesticide Sub-Group of CORESTA has been testing analytical methods to quantify the residues of Pendimethalin on tobacco. This exercise has been carried out conjointly with the Agronomy Group, which has organized the application of the chemical in the field and the preparation of the samples. As the results reflect all types of dispersions (field treatment, differing climates, influences during and after treatment, sampling, inter- and intra-laboratory biases), no statement about a precision of the usual type can be made. However, an estimation of the precision of the method via the coefficients of variation at different residue levels will be given.

Three parameters seemed to be decisive for the usefulness of the method, (1) Reliability of the results, (2) Simplicity of the analytical procedure and (3) Low-cost equipment. The majority of the Pesticide Sub-Group was in favour of a method which was adapted by Len Toet of the Tobacco Research Board, Harare, Zimbabwe, for the needs of this exercise. Valuable details were added by group members (Norman Lorton, Rothmans UK). The figures given in this report represent the results of the Joint Experiment JE-25 (samples by V. Kozumplik, Tobacco Institute, Zagreb, Yugoslavia) and JE-27 (samples by Len Toet, Tobacco Research Board, Harare, Zimbabwe).

1. SAMPLE PREPARATION

1.1. Weigh 10 g of ground tobacco and extract for six hours in a soxhlet extractor with hexane (volume greater than 100 cm^3). Dry the sample by passing it through 10 g of sodium sulphate (Na_2SO_4) and a cotton wool plug.

1.2. Evaporate to 50 cm^3 on a rotary evaporator (temperature 60°C).

1.3. Place 25 cm^3 of the sample in a separating funnel and extract three times with 15 cm^3 acetonitrile (methyl cyanide, CH_3CN).

1.4. Evaporate to dryness on a rotary evaporator (temperature 60°C).

1.5. Add 10 cm^3 hexane and transfer to a stoppered measuring cylinder.

1.6. Wash until the total volume is 20 cm^3.

1.7. Take a 10 cm^3 aliquot and pass it through a short, untreated Florisil column.

1.8. Elute with 2 x 10 cm^3 hexane and discard. Elute with 50 cm^3 hexane/diethylether (9+1) and collect the eluate.

1.9. Evaporate to dryness and make up the volume to 50 cm^3 with hexane. Make up to 5 cm^3 if this is necessary for low concentrations.

1.10. Inject a 5 mm^3 aliquot into the GC. Suitable GC conditions see under 2.

Spiking : The recovery of this method can be checked by spiking the tobacco sample at the 1 ppm (1 µg/g) level.

2. SUITABLE GC CONDITIONS

2.1. Packed column :

Length:	1 m
Material:	11% OV17 - QF1 (1+1)
Column temperature:	225°C
Injector temperature:	235°C
Detector type:	ECD
Detector temperature:	300°C
Gas flow:	50 cm^3/min (N_2 or Ar/CH_4 95+5)

The calibration standards should be in the range of 10^{-7} g/cm^3. Injection volume 1 - 5 mm^3.

2.2. Capillary column :

Dimensions:	50 m x 0.32 mm i.d.
Type:	WCOT fused silica
Stationary phase:	CP-SIL-19 CB
Film thickness:	0.19 µm
Carrier gas:	Helium
Inlet pressure:	175 kPa
Injector type:	Split/splitless
Injection mode:	Split
Temperature:	280°C
Split-flow:	60 cm^3/min
Detector type:	ECD
Make-up gas:	Nitrogen
Make-up flow:	30 cm3/min
Temperature:	300°C
Oven program:	Start at 150°C with 10°C/min to 280°C, then 11 min at 280°C

3. ASSESSMENT OF RESULTS

3.0. This assessment uses a total of 35 results which were contributed by five laboratories.

3.1. Recovery :

The recovery can vary from 82-105% (mean of five laboratories: 87%) and should always be determined during the analysis of each batch of samples.

3.2. Blank value :

Based on 11 results of 4 laboratories, a blank value of 0.3 ppm Pendimethalin on untreated leaves was determined (coefficient of variation 29,0%). These results could include a possible carry-over via the soil or from treatments of adjacent fields.

3.3. Range :

The method was tested for a range of around 1 ppm of residue. It seems that with this type of treatment (1 x and 3 x the recommended concentration), no higher residue can be found.

3.4. Overall precision of the method (see foreword) :

The coefficient of variation (standard deviation expressed as percentage of mean value) shows a dependency on the residue level. It was estimated as 30/70% for 0.1 - 0.5 ppm, 20% for 0.5 - 1.0 ppm, and 15% above 1.0 ppm.

CORESTA RECOMMENDED METHOD N° 32

DETERMINATION OF RESIDUES OF THE SUCKERCIDE OFF-SHOOT-T (N-ALKANOL MIXTURE) ON TOBACCO

(June 1991)

0. FOREWORD

During the last years, the Pesticide Sub-Group of CORESTA has been testing analytical methods to quantify the residues of Off-Shoot-T on tobacco. This exercise has been carried out conjointly with the Agronomy Group, which has organized the application of the chemical in the field and the preparation of the samples. As the results reflect all types of dispersions (field treatment, differing climates, influences during and after treatment, sampling, inter- and intra-laboratory biases), no statement about a precision of the usual type can be made. However, an estimation of the precision of the method via the coefficients of variation at different residue levels will be given.

Three parameters seemed to be decisive for the usefulness of the method, (1) Reliability of the results, (2) Simplicity of the analytical procedure and (3) Low-cost equipment. The majority of the Pesticide Sub-Group was in favour of a method which was published by K. Polzhofer, Unilever Forschungsgesellschaft in Z. Lebensm. Unters.-Forsch. 164, 21-22 (1977). (Some paragraphs of this method will be reprinted hereunder in an abridged version). The figures given in this report represent the results of the Joint Experiments JE-24 (samples by A. Beuchat, Deltafina, Italy) and JE-25 (samples by V. Kozumplik, Tobacco Institute, Zagreb, Yugoslavia).

1. SAMPLE PREPARATION

1.1. Thaw the samples, which have been stored at -20°C, at room temperature and grind finely.

1.2. Transfer 20 g of the sample with 300 cm^3 of diethyl ether to a conical flask containing approximately 10 g of glass beads and shake for 1 hour.

1.3. Filter the mixture over glass wool and centrifuge the filtrate at 0 - 10°C at 10,000 rpm for 20 min.

1.4. Evaporate 225 cm^3 of the extraction solution, corresponding to 15 g of substrate, to approximately 2 cm^3 at a pressure of about 1.33 kPa and at a temperature no higher than 40°C in a rotary evaporator. (Caution: do not evaporate to dryness!).

1.5. Add 1 g of Celite 545, 4 g of sea-shore sand and 6 g of granulated Na_2SO_4 to the concentrate and grind in a mortar.

1.6. Suspend 5 g of Florisil (5% of water) in n-hexane in a chromatography tube closed with glass wool. Pour the free-flowing mixture onto the column packing.

1.7. Elute with 150 cm^3 of each of the following eluents:
a) n-hexane, b) n-hexane/dichloromethane (8 + 2).

1.8. Combine the eluates and evaporate to approx. 1 cm^3 at a pressure of approximately 1.33 kPa and a temperature no higher than 40°C. (Caution: do not evaporate to dryness!).

1.9. Make up the resulting concentrate to 2.0 cm^3 with dichloromethane.

1.10. Use approx. 1-5 mm^3 of each of the solutions for the gas chromatographic determination.

2. SUITABLE GC CONDITIONS

Equipment :

Hewlett-Packard 5700 A gas chromatograph (or equivalent)
FID detector

Packed column material :	10% DC-200 on Gaschrom Q (451)
Hydrogen/Air flow rate (FID) :	40/240 cm^3/min
Temperature of injection block :	250°C
Temperature of detector :	300°C
Temperatureof column :	130°C for 11 min then 8°C/min to 230°C
Carrier gas flow rate (N_2) :	25 cm^3/min
Column geometry :	1.8 m x 2 mm i.d.; glass coil
Chart recorder :	1 mV, 0.635 cm/min (0.25 inch/min)
Retention times :	octan-1-ol: 2.2 min, decan-1-ol: 6.5 min

3. ASSESSMENT OF RESULTS

3.0. This assessment uses a total of 54 results which were contributed to these two joint experiments.

3.1. Recovery :

The recovery varies from 85-104% and should always be determined during the analysis of a batch of samples.

3.2. Range :

The method was tested for a range of around 0-2 ppm of residue. It seems that with this type of treatment (recommended concentrations), no higher residues can be found.

3.3. Overall precision of the method (see foreword) :

The coefficient of variation (standard deviation expressed as a percentage of mean value) as given by K. Polzhofer shows a dependency on the residue level. It was estimated as 100%, 50%, 12.5% for residue levels of 0.01 ppm, 0.1 ppm, 1.0 ppm and 10.0 ppm respectively.