Rhône-Poulenc Ag Company
Standard Operating Procedure

SUMMARY

Residues of fipronil and its metabolites MB 45950, MB 46513, RPA200766 and MB46136 are extracted from the soil with acetonitrile / acetone (70/30 v/v) mixture. After shaking, the soil sample is centrifuged and a portion of the extract is dried with sodium sulfate, the analytes are adsorbed onto granular charcoal, then eluted with acetonitrile. The eluant is then concentrated to the desired volume before analysis with an Electron Capture Gas Chromatograph equipped with a DB-1701 capillary column.

1. Safety: All procedures must be conducted in compliance with the safety regulations of Rhône Poulenc Ag Company.

2. EXPERIMENTAL

2.1 Reagents

Acetone, HPLC grade, Burdick & Jackson.
Acetonitrile, HPLC-UV grade. Burdick & Jackson.
Activated carbon, darco 20-40 mesh. Aldrich (cat # 24,226-8).
Sodium sulfate, anhydrous, Aldrich.
Fipronil, MB 46030, Rhône-Poulenc.
MB 45950, Rhône-Poulenc.
MB 46513, Rhône-Poulenc.
MB 46136, Rhône-Poulenc.
RPA 200766, Rhône-Poulenc.

Appendix B

2.2. Chromatographic Conditions

Instrument : Gas chromatograph (HP 5890):

Column:	J&W, DB-1701, 15 M x 320 µM i.d., 0.25 µM film thickness (part # 123-0712).
Detector:	Ni^{63} Electron Capture.
Oven Temperature:	Initial temp 50 °C., hold for 1 minute. Program ramp 70 °C/minute to 200 °C. Hold for 22 minutes. Ramp 70 °C/minute to 230 °C and hold for 17 minutes
Injection Temperature:	280 °C.
Detector Temperature:	300 °C.
Flow Rates:	
Helium (carrier)	1.9 ml/min.
95% Methane/Argon (make-up)	50 ml/min.
Integrator:	Hewlett-Packard HP3396 series 2 or equivalent. Due to the sharp temperature rates used in this program, the integrator must be instructed to zero to the baseline when necessary.

Waters 860 Networking Computer System, version 3.0, or equivalent.

Typical retention Times:		
	MB 46513	10.5 minutes
	MB 45950	15 minutes
	MB 46030	16 minutes
	RPA 200766	30 minutes
	MB 46136	31 minutes

2.3. Apparatus

1. Mettler PM4600 balance or equivalent.
2. Mettler AE163 balance or equivalent.
3. N-Evap, model 106, Organomation Assoc. Inc.or equivalent.
4. Beckman model TJ-6 centrifuge.or equivalent.
5. Adlab Shaker, Arthur H. Thomas Company, Philadelphia, PA, USA or equivalent.

2.4. Preparation of Charcoal Columns

Pack a 10 mL Bio-Rad Poly-Prep chromatography column with approximately 2 g of granular activated carbon (20-40 mesh). Although the activated carbon can be used as purchased from the manufacturer, it is recommended that the carbon be heated at approximately 120 °C over night to prevent moisture. Top with approximately 2-3 g granular anhydrous sodium sulfate. Columns similar to the 10 ml Bio-Rad chromatography columns could be used for this procedure. Open columns should be plugged with a piece of glass wool before packing with charcoal.

2.5. Preparation of standard and spiking solutions

Weigh 100 mg of each of MB 46030, MB 45950, MB 46513, RPA200766 and MB46136 individually into 100 ml volumetric flasks. Dissolve each compound in approximately 50 ml of acetonitrile and bring up the volume to the mark with acetonitrile. This is now 1000 µg/ml solution of each individual standard. Transfer 1 ml of each solution to a 100 ml volumetric flask using 1 ml pipets and bring up the volume to the mark with acetonitrile. This solution is 10 µg/ml of each compound as combined standard. Using this stock solution, make the appropriate dilutions to prepare 1 µg/ml, 0.5 µg/ml, 0.1 µg/ml 0.02 µg/ml, 0.01 µg/ml 0.008 µg/ml, 0.005 µg/ml and 0.002 µg/ml solutions in acetonitrile. All dilutions must be made using grade A glassware. Use these solutions as standards and soil fortification solutions.

The 1000 µg/ml solutions could be prepared as a mixture by combining the weights of all five analytes in one volumetric flask.

2.6. Extraction and Cleanup Procedure:

1. Weigh 50 g soil into a 250 mL Nalgene screw-capped bottle.
   Fortification of control samples for the determination of recoveries should be done at this point.
2. Add 100 mL 30% acetone in acetonitrile (UV grade) and shake vigorously by hand for one minute. Place bottle in mechanical shaker and shake for 30 minutes.

3. Centrifuge for 5 minutes.

4. Rinse a carbon/Na_2SO_4 column above with approximately 10 mL methanol, 10 mL acetone, then 10 mL of UV grade acetonitrile.

5. Place an Erlenmeyer flask under the column. Pipet a 4 mL aliquot from the soil sample and put onto the charcoal column. Rinse the column with approximately two 5 mL portions of acetonitrile.

6. Connect a 20 mL polypropylene disposable column to the top of the charcoal column. This is used as a reservoir only.
Add an additional 40-45 mL UV grade acetonitrile.

7. Place the Erlenmeyer in water bath, not to exceed 50 °C and evaporate to approximately 3 mL using a gentle stream of nitrogen.

8. Transfer to a 15 mL graduated centrifuge tube, rinsing the Erlenmeyer several times with acetonitrile.

9. Place the centrifuge tube in an N-EVAP analytical evaporator (or equivalent) and evaporate to desired volume. Any necessary dilutions should be done at this point.

2.7 GC Resolution and Retention Times

Typical retention times obtained using the conditions described in section 2.2 were as follows MB 46513 (10.5 minutes), MB 45950 (15 minutes), MB 46030 (16 minutes), RPA 200766 (30 minutes), MB 46136 (31 minutes). The DB-1701 capillary column and the chromatographic conditions used gave over 1 minute separation of MB 46030 and MB 45950. It also gave about 1 minute separation of RPA 200766 and MB 46136. These separations were not possible using a variety of other capillary columns.

2.8. Quantification of Residues

1. The residues of fipronil and its metabolites in the extracts should be quantified by comparison with standard solutions of appropriate concentration (standard curve). This should be done utilizing the Waters 860 Networking Computer System, version 3.0, or equivalent. The formula and an example of the calculations performed by this system are given below. In cases when this system is not operating, the integrator output could be accepted as raw

data. In this case the linear regression and the residues should be calculated with a scientific calculator capable of performing this operation..

2. When necessary, the extracts should be diluted so that the peak areas or heights obtained are within the linear range of the detector (0.002-0.02 µg/ml)..

Equation used in the calculations:

$$PPB = \frac{(Y-A)}{B} \times \frac{F}{W}$$

where:

PPB= Parts per billion
Y = Peak area
A = Intercept of standard curve
B = Slope of standard curve
F = Scale factor (final volume in milliliters)
W = Sample weight

Note: The 4 ml aliquot of the sample extract analyzed represents 2 grams of soil since 50 grams of soil are extracted with 100 ml of solvent.

Example of calculations:

The following example show the calculations of the concentration of fipronil (MB 46030) in soil spiked with 10 ppb of each of the five compounds under analysis.

Peak area = 27128 area counts
Intercept of the standard curve = 1822 area counts
Slope of the standard curve = 4467 counts/ng/ml
Scale factor (final volume) = 2 ml
Sample amount = 2 grams

$$PPB \text{ of fipronil} = \frac{(27128 \text{ counts} - 1822 \text{ counts})}{2607 \frac{\text{counts}}{\text{ng/ml}}} \times \frac{2 \text{ ml}}{2 \text{ g}} = 9.7 \text{ ppb}$$

The chromatograms of the sample and standards used in these calculations are shown in the appendix provided.

2.9 Recovery Determinations

Two spikes should be performed with each set, a low spike (5 or 10 ppb) and a high spike (100 ppb). In addition to these routine spikes, other spikes that exceed the highest level of residue found in the samples must also be performed.

Spike recoveries should be calculated using the following formula.

$$\% \text{ recovery} = \frac{\text{ppb found}}{\text{ppb spiked}} \times 100$$

Example:

From the calculations given above, the concentration of fipronil in the spiked sample as calculated is 9.7 ppb. The concentration of fipronil spiked is 10 ppb, therefore.

$$\% \text{ recovery of fipronil} = \frac{9.7}{10} \times 100 = 97\%$$

CRITICAL PPOINTS

1. Any filtration steps are not recommended because of possible interferences from the filter units. If filtration becomes necessary, it should be done using glass fiber filters.

2. Reconditioning of the capillary column becomes necessary after several injections of soil extracts. Reconditioning of the column can be done by breaking about twenty centimeters from the front of the column. To avoid having to break the column a retention gap could be used.

3. The purity of acetonitrile should be checked before used in the analysis. This purity check can be done by taking approximately 40 ml of acetonitrile and concentrating it to 2 ml using nitrogen evaporator (N-Evap) and injecting the concentrated solvent on GC.