Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Fipronil

MRID #: 451359-02

Matrix: Water

Analysis: LC/MS/MS

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451359-02

Title: Insecticides, Fipronil: Method of Analysis for Possible Residues of Fipronil, MB46513, MB45950, and MB46136 in Water.

Analytes: Fipronil, MB46513, MB45950, and MB46136.

Substrates: Water

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Method of Analysis for Fipronil
and Its Metabolites in Water

I. INTRODUCTION

A. Scope

This method sets forth the procedure for determining the residues of fipronil (MB46030) and its metabolites MB45950, MB46136 and MB46513 in surface water

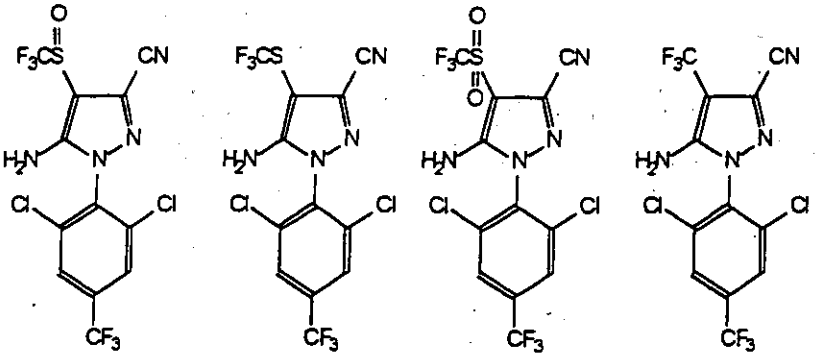
B. Principle

An analytical method is described for the determination of residues of fipronil and its metabolites MB45950, MB46136 and MB46513 in surface water. Following addition of acetonitrile to produce a final ratio of 20% acetonitrile:water, residues of fipronil and its metabolites MB45950, MB46136 and MB46513 in surface water are determined by direct injection onto the LC/MS/MS system. Quantification of results is based on a comparison of peak areas with those of known standards.

C. Method Limits

The limit of detection (LOD) and limits of quantification (LOQ) for fipronil and its metabolites MB45950, MB46136 and MB46513 in water have not been determined. This information will be obtained from the subsequent validation study. Target level LOQ is 10 ppt.

D. Structures of Test Substances



MB 46030 (Fipronil) MB 45950 MB 46136 MB 46513

II. Materials

Unless otherwise noted, equivalent brands and/or suppliers can be used.

A. Reagents/Solvents

Acetic acid GR (EM Science Cat. No. AX0073)

Acetonitrile Omni-Solv (EM Science, Cat. No. AX0142)

Water Omni-Solv, HPLC Grade (EM Science, Cat. No. WX0004)

B. Equipment and Supplies

Balance :

accuracy ± 0.1 mg (analytical standards), Mettler AT 201 or equivalent
accuracy ± 0.1 g (samples and chemicals), Mettler PC 4000 or equivalent

Disposable pipettes

Micropipetter, VWRbrand (Calibra) and pipet tips.

Graduated polyethylene bottles (Dynalab Corporation, catalog number 2629).

Graduated cylinders

Pipette bulb

Solvent jugs, 4 L brown glass

Volumetric flasks

Volumetric pipettes

Vial, clear, 1.5mL; cap, open top; septa, split (Sun, 200-250; 200-292, 500-870)

Note: Because of the possibility of Fipronil residue adhering to glass surfaces, all glassware used in the method should be cleaned according to the following steps.

1. Wash the glassware in hot, soapy tap water.
2. Rinse well with hot tap water.
3. Rinse again with deionized water.
4. Rinse a third time with methanol or acetone.
5. Dry completely on a rack.
6. Place in the muffle oven.
7. Muffle Oven: Barnstead/Thermolyne, model F53700 or equivalent.

   Muffle Oven Settings:
   Set point 0 = 25 °C, Ramp to set point 1 (450 °C) in 1 hour. Hold at set point 1 (450 °C) for 3 hours. Cool to 25 °C in 2 hours.

C. Solutions

The following is a list of the solutions used in the analyses of surface water. Example procedures for the preparation of each solution are also provided. Note that the reagent water used in the preparations should be HPLC grade.

1. Solution of 20:80 acetonitrile:water.
   Using a graduated cylinder, transfer 800 mL H2O and 200 mL CH3CN to a 4 L brown glass solvent jug that is clean and dry or a jug which previously contained acetonitrile.

2. ~1.5% Acetic acid Water
   Using a graduated cylinder, transfer ~985 mL of HPLC grade H2O and to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Pipet 15 mL acetic acid and transfer to the same container. Repeat until the desired quantity has been made.

III. FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

A. Preparation

All the standard solutions must be stored in amber glass bottles. Standard solutions will be stored at 5°C ± 3°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example of a procedure to follow in preparing standard solutions. Alternate or additional standards of appropriate weight and volume may be prepared as needed. The "~" symbol indicates approximately.

Stock solutions:

1. Weigh ~0.1000g (corrected for purity) each of fipronil, MB46136, MB46513 and MB45950 into separate 100mL volumetric flasks and dilute to the mark with acetonitrile. Sonicate for approximately 5 minutes if necessary. The concentration of these stock standards is ~1000µg/mL of fipronil, MB46136, MB46513 and MB45950.

Mixed Standards Solutions:

2. Transfer 10mL of the ~1000µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 50:50 solution of 0.1%acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~100µg/mL of fipronil, MB46136, MB46513 and MB45950.

3. Transfer 10mL of the ~100µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 50:50 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~10µg/mL of fipronil, MB46136, MB46513 and MB45950.

4. Transfer 10mL of the ~10µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 50:50 solution of 0.1%acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~1.0µg/mL of fipronil, MB46136, MB46513 and MB45950.

5. Transfer 10mL of the ~1.0µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~0.1µg/mL of fipronil, MB46136, MB46513 and MB45950.

6. Transfer 20mL of the ~0.1µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1%acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~20ηg/mL of fipronil, MB46136, MB46513 and MB45950.

7. Transfer 10mL of the ~0.1µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~10ηg/mL of fipronil, MB46136, MB46513 and MB45950.

8. Transfer 5mL of the ~0.1µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~5.0ηg/mL of fipronil, MB46136, MB46513 and MB45950.

9. Transfer 3mL of the ~0.1µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~3.0ηg/mL of fipronil, MB46136, MB46513 and MB45950.

10. Transfer 1mL of the ~0.1µg/mL fipronil, MB46136, MB46513 and MB45950 standard solutions, via volumetric class "A" pipettes, to one 100mL volumetric flask. Dilute to mark with a 80:20 solution of 0.1% acetic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~1.0ηg/mL of fipronil, MB46136, MB46513 and MB45950. Using this stock solution, make the appropriate dilution to prepare 0.1ηg/mL, 0.080ηg/mL, 0.06ηg/mL, 0.04ηg/mL, 0.03ηg/mL, 0.02ηg/mL, 0.01ηg/mL, 0.005ηg/mL, etc. solutions. All dilution must be made using grade A glassware. Use these solutions as standards and fortification solutions.

B. Stability

Calibration standard and fortification solutions of fipronil and its metabolites (MB 45940, MB 46136, MB 46513 and RPA 200766) in acetonitrile have been shown to be stable for periods of approximately three months when stored within a temperature range of 0°C to +4°C ("Fipronil: Magnitude of Residues in/on Cottonseed and Gin Trash Under a Modified Application Scenario", Study No. US95V10R, Robert S. Plaisance, Dec. 10, 1996; "Fipronil: Validation of Method of Analysis for Fipronil and its Metabolites in Field Corn", Study No. EC-93-236, Suvit Upalawanna, Aug. 27, 1993 (MRID #43323401).

IV. Method Procedures

A. Analysis of Ground and Surface Waters

(Analysis for Fipronil, MB46136, MB46513 and MB45950 by direct injection)

1. Water samples are retrieved from the crop room refrigerator and allowed to warm to room temperature.

2. Transfer the entire water sample into a graduated polyethylene container (suitable for the volume of the water sample and the addition of 25% acetonitrile with some room left for mixing the sample). Record the volume of the water sample

   Note: at this point, if multiple water samples, of approximately the same volume, are being analyzed in one set, the original volumes of the of the water samples can be brought up to one common volume with HPLC grade water. For example, if the water samples being analyzed are 900mL, 950mL, 980mL, 975mL, 960 mL, then, as a matter of convenience, these samples can be made up to 1000mL. The original sample volume, however, must be recorded for accurate calculation of the residues in the original water sample. Care must be taken not to significantly dilute the water samples to the point were low level residues could not be detected. Samples should not be diluted more than approximately 30%.

3. Obtain a volume of acetonitrile equivalent to 25% of the total water sample

4. Add approximately half of the acetonitrile to the original sample container, cap the container and hand shake for one minute, then place on a mechanical shaker and shake for approximately 5 minutes to dissolve any fipronil residues bound to the inner surface of the container. Transfer the acetonitrile to the graduated polyethylene bottle..

5. Repeat step 4 with the second portion of acetonitrile and transfer to the graduated polyethylene bottle.

6. Cap the graduated bottles and mix well by shaking for 1 minute, then place on a mechanical shaker and shake for 10 minutes..

7. Using a disposable pipette an aliquot of the acetonitrile/water mixture is drawn from the graduated cylinder and placed directly in an HPLC vial. Vials must be properly labeled.

8. HPLC grade water is used for the untreated control. The water should be poured into a clean disposable beaker.

9. A known amount of HPLC water similar to the volume of the water samples being analyzed is used for the recovery sample and spiked with a known amount of a fortification solution to give the desired level of fortificatio.

10. Analyze samples by LC/MS/MS under the conditions described in Section V.

V. Chromatographic System

LC/MS/MS System	Perkin Elmer Sciex API 3000 LC/MS/MS system with PE Sciex Turbo Ionspray Electrospray Interface Shimadzu LC-10AD VP HPLC pumps (2) with 250 µL high pressure mixer and SCL-10A VP Pump Controller. Perkin Elmer Series 200 autosampler
Ionization and MS Mode:	Electrospray (TurbolonSpray) - negative ion mode
MS Mode:	MS/MS with multiple reaction monitoring (MRM)
Ion Spray / Orifice Voltage:	-5000V / -60V
Nebulizer Setting:	15 (Air)
Curtain Gas Setting:	9 (Nitrogen)
Turbo IonSpray Settings:	Heated air at ~8.5 L/min, 500°C
Collision Gas Setting:	8 (Nitrogen)
Collision Energy (see below):	R02-Q0, Q0=6V

Mass Transitions, Collision Energy (Dwell Times 250ms):

Fipronil:	435/330amu,	25-6 = 19V
MB46513:	387/351	26-6 = 20V
MB45950:	419/383	25-6 = 19V
MB46136	451/415	32-6 = 26V

Column: Use two of the following, closely coupled, columns: YMC ODS-AQ, 2.0 X 50mm, 3µm particle size, 120A pore size. These two columns can be

substituted with one 2.0x100mm column of the same type.

Mobile Phase Flow Rate:	0.250mL/min, no split (A valve may be used to divert mobile phase containing only salts and matrix away from the mass spectrometer before peaks of interest elute.)
Mobile Phase Composition:	70% Acetonitrile / 30% (1.5% Acetic Acid in Water)
Injection Volume:	35µL, (can be increased if needed)
Retention times:	See chromatograms

Note the indicated LC-MS-MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

VI. CALCULATIONS

Linear regression should be used to generate calibration curves for fipronil, MB46136, MB46513 and MB45950. After the instrument performance criteria are met, a minimum of four standards over a range of concentration levels should be included with a set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Samples should be diluted such that any peak areas or heights are within the area or height range between the lowest and highest standards injected.

Linear regression coefficients should be calculated on standard concentration (ng/mL) versus peak area or height. The data from the analytical standards should then be fit to the linear model,

$$Y = A + BX.$$

The equation to be used to estimate the residues in the samples is:

$$E = \frac{(Y - A) \times C}{B \quad D}$$

where: Y = response of analyte of interest (peak area or height)
A = intercept from linear regression analysis (peak area or height)
B = slope from linear regression analysis (response per concentration)
C = final sample volume (mL)
D = starting weight in grams of sample in final volume (g)
E = concentration of analyte in sample in parts per billion (ppb or ng/mL)

VII. SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the study. General laboratory safety precautions should be taken. This method does not present any specific risks.

VIII. REFERENCES

"Fipronil: Magnitude of Residues in/on Cottonseed and Gin Trash Under a Modified Application Scenario", Study No. US95V10R, Robert S. Plaisance, Dec. 10, 1996; "Fipronil: Validation of Method of Analysis for Fipronil and its Metabolites in Field Corn", Study No. EC-93-236, Suvit Upalawanna, Aug. 27, 1993 (MRID #43323401).

Example Chromatogram: 0.02 ηg/mL standard