

## ANALYTICAL METHOD FOR THE DETERMINATION OF SELECTED SOIL METABOLITES OF PROPACHLOR IN FIELD SOIL SAMPLES

## I. SUMMARY AND INTRODUCTION

## A. Scope

The following method has been developed to determine levels of three soil metabolites of propachlor in field soil samples. The structures of I, II, and III are shown in Figure 1.

- I. [{}((methylethyl)phenylamino)acetyl]sulfinyl]-acetic acid]
- II. [(1-methylethyl)phenylamino]-2-oxoacetic acid]
- III. [2-((1-methylethyl)phenylamino)-2-oxoethane-sulfonic acid]

## B. Summary

The analytical method described can be used for the determination of three soil metabolites of propachlor: I, II, III. Soil samples are first extracted with acetonitrile/water in order to initially isolate the three weakly acidic metabolites. Next, the three metabolites are isolated from the extract by High Performance Liquid Chromatography (HPLC) operated isocratically in a reverse phase mode using a semi-preparative size octylsilane-derivatized column. Individual fractions containing each of the three metabolites are collected using a micro-processor controlled fraction collector. Final analytical determination is accomplished by reversed phase ion-pairing HPLC using an analytical octadecylsilane column and ultraviolet spectrophotometric detection. Fractions containing I, II, and III can be either composited or analyzed separately.

A "Set of Samples" consists of duplicate samples from each treatment rate at each sampling collection date, two check (untreated) samples, and two check samples fortified at a specific level with each of the three soil metabolites. All samples, i.e., field samples, check samples, and fortified samples, are subjected to HPLC cleanup with fraction collection. Collection windows for the metabolites are checked before and after a sample set is run so as to confirm complete collection of the

correct peaks. Once collected, fractions are ready for final determination using the ion-pairing HPLC/UV.

Analytical recoveries are estimated for I, II, and III based upon the the range of recoveries of the three metabolites from samples spiked across the anticipated environmental levels. Upon completion of all analyses, recoveries of I, II, and III from the 0-6" layer and the 6-12" layer are pooled and average recoveries for each analyte are calculated. The average recoveries are then used to correct the levels of I, II, and III. A flow chart summarizing the method is shown in Figure 2.

### C. Detectability

The lower limit of method validation (LMV) of this procedure is 0.050 ppm for each of the three acidic metabolites. The limit of detection (LOD) as estimated from the background response of check samples for each metabolite was found to be 0.051 ppm, 0.049 ppm, and 0.056 ppm for metabolites I, II, and III, respectively. These values are only slightly above the 0.050 ppm limit of method validation for each metabolites and may be artifically high due to outliers in the data used to calculate the LOD (see the Accuracy and Precision). Therefore, all values in the study were reported down to the LMV of 0.050 ppm, for each of the three metabolites.

## II. MATERIALS/METHODS

### A. Equipment

1. Balance, Mettler PE3600 or equivalent
2. Balance, Mettler AE135 or equivalent
3. Vacuum rotary evaporators equipped with water baths
4. Hot plates, Fisher No. 11-493-200T
5. Pipets, graduated and disposable, Fisher Nos. 13-676-28(A-G)
6. Volumetric flasks, 100 mL, Fisher No. 10-210C
7. Nylon 66 luer-lok filters, 0.45 microns, Millipore no. SLHV025NB

8. Disposable syringes, 3 mL, Fisher No. 14-823-39
9. Culture tubes, 16 X 100 mm, Wheaton No. 358646
10. Pasteur pipette, 5 3/4" length, Fisher No. 13-678-6A
11. Pasteur pipette, 9" length, Fisher No. 13-678-6B
12. Vials, Varian No. 66-000104-00
13. Teflon septa, IBM No. 8635463
14. Caps for IBM autosampler, IBM No. 8635455
15. Chart recorder, Fisher Series 5000
16. Chart paper, Fisher No. 13-939-35
17. IBM 9533 Tertiary Gradient HPLC
18. IBM 9505 Autosampler
19. Spectroflow 757 Variable wavelength UV detector
20. Gilson 202 fraction collector
21. HPLC Zorbax C8 semi-prep column, 25 cm X 9.4 mm ID, Fisher No. 06-642-2J
22. HPLC Zorbax C18 column, 25 cm X 4.6 mm ID, Fisher No. 06-642-1H
23. Perkin-Elmer LC-95 Spectrophotometric Detector
24. Perkin-Elmer ISS-100 autosampler
25. Varian 2010 HPLC Pump
26. Systec column heater, CH 1448, dual zone temperature controller
27. Roundbottom flasks, 100 mL and 500 mL, Fisher No. 10-067-2B and F
28. Teflon lined 4L beaker, Fisher No. 02-556-L
29. Orion ionanalyzer EA 920 or equivalent
30. Glaspun cloth tape, Fisher No. 01-472B

31. Filter flasks, 4000 mL Fisher No. 10-181H
32. Nylon 66 0.22 micron filters, Fisher No. NO2-SP047-00
33. Suction tube adapters, Fisher No. 15-323C
34. Graduated cylinder, 2000 mL, Fisher No. 08-552-1G
35. Glass fiber filter papers, Fisher No. 9-873
36. French square bottles, 16 oz. with caps, Northwestern Bottle
37. Guard column cartridge, 12.5 X 4 mm, Dupont No. 820674-905
38. Fittings kit for guard column, Dupont No. 820529-901
39. Buchner funnels, Fisher No. 10-356
40. Graduated cylinders with penny stopper heads, Fisher No. 08-566C
41. Mohr pipettes, 25 mL, Fisher No. 13-666-1H
42. Mechanical shaker
43. Vortex mixer
44. Stir bars

B. Solvents, Reagents, and Standards

1. Acetonitrile, Fisher No. A-998-4
2. Methanol, Fisher No. A-452-4
3. Formic acid 88%, Fisher No. A-118
4. Ammonium hydroxide, Fisher No. A669-500
5. Water, HPLC grade, Milli-Q
6. Dodecyltriethyl ammonium phosphate, Regis Chemical No. 404021
7. Potassium phosphate, HPLC grade, Fisher No. P-236

8. Sodium hydroxide, 50% w/w, Fisher No. SO-S-254
9. Buffer, pH 4, Fisher No. SO-B-98
10. Buffer, pH 7, Fisher No. SO-B-108
11. [(1-methylethyl)phenylamino]-2-oxoacetic acid, analytical standard, purity >95%
12. 2-[(1-methylethyl)phenylamino]-2-oxoacetic acid, analytical standard, purity >95%
13. [[[methylethyl)phenylamino]acetyl]sulfinyl]-acetic acid, analytical standard, purity >95%

#### C. Standard Solutions

##### 1. Spiking Solutions

Weigh 0.1000 grams of I into a volumetric flask, dilute to volume with methanol, and mix well to insure complete dissolution. This solution contains 1000  $\mu\text{g/mL}$  of I.

Weigh 0.1000 grams of II into a volumetric flask, dilute to volume with methanol and mix well to insure complete dissolution. This solution contains 1000  $\mu\text{g/mL}$  of II.

Weigh 0.1000 grams of III into a volumetric flask, dilute to volume with methanol and mix well to insure complete dissolution. This solution contains 1000  $\mu\text{g/mL}$  of III.

Pipette 10.0 mL of I, II, and III respectively into a 100 mL volumetric flask, dilute to volume with methanol and mix thoroughly. This standard contains 100  $\mu\text{g/mL}$  of I, II, and III.

Pipette 10.0 mL of the 100  $\mu\text{g/mL}$  standard into a 100 mL volumetric flask, dilute to volume with methanol and mix thoroughly. This standard contains 10.0  $\mu\text{g/mL}$  of I, II, and III.

Standards are stored in amber glass bottles at 2° to 5°C.

##### 2. HPLC/UV Standards

Pipette 0.10 mL of the 1000  $\mu\text{g/mL}$  fortification standard into a 100 mL volumetric flask.

Dilute to volume with fresh ion pairing reagent prepared that day for the samples to be analyzed. Mix well. This external standard contains 1.00  $\mu\text{g/mL}$  of I, II, and III.

Pipette 25.0 mL of the 1.00  $\mu\text{g/mL}$  solution into a 50.0 mL graduated cylinder with a penny stopper head. Pipette an additional 25.0 mL of fresh ion pairing reagent prepared that day for the samples to be analyzed. Mix well. This external standard contains 0.50  $\mu\text{g/mL}$  of I, II, and III.

Pipette 25.0 mL of the 0.50  $\mu\text{g/mL}$  solution into a 50.0 mL graduated cylinder with a penny stopper head. Pipette an additional 25.0 mL of fresh ion pairing reagent prepared that day for the samples to be analyzed. Mix well. This external standard contains 0.25  $\mu\text{g/mL}$  of I, II, and III.

Pipette 20.0 mL of the 0.25  $\mu\text{g/mL}$  solution into a 50.0 mL graduated cylinder with a penny stopper head. Pipette an additional 30.0 mL of fresh ion pairing reagent prepared that day for the samples to be analyzed. Mix well. This external standard contains 0.10  $\mu\text{g/mL}$  of I, II, and III.

Pipette 10.0 mL of the 0.10  $\mu\text{g/mL}$  solution into a 50.0 mL graduated cylinder with a penny stopper head. Pipette an additional 40.0 mL of fresh ion pairing reagent prepared that day for the samples to be analyzed. Mix well. This external standard contains 0.02  $\mu\text{g/mL}$  of I, II, and III.

It is important that the ion pairing solution used for final sample dilution, external standards, autosampler reservoir, and mobile phase be identical in composition, i.e., they come from the same stock solution. Failure to do so will contribute to negative peaks in the baseline and may interfere with peak quantitation.

## D. Reverse Phase HPLC Operating Conditions

Column: Zorbax C8 bonded phase, 25 cm x 9.4 mm ID, 5 micron particle size

Column temperature: 35°C

Flow rate: 1.2 mL/minute

Mobile phase: 23% Acetonitrile  
77% 0.54 N Ammonium formate buffer  
adjusted to pH 3.8 with NH<sub>4</sub>OH

Injection volume: 1000 µL

Wavelength: 235 nm

Chart: 10 mV full scale

Attenuation: 0.1 AUFS

Response time: 1 sec filter rise time

Run time: 40 minutes

## E. Ion Pairing HPLC Operating Conditions

Column: Zorbax C18 bonded phase, 25 cm X 4.6 mm ID, 5 micron particle size

Column temperature: ca. 45°C

Flow rate: ca. 1.0 mL/minute

Mobile phase: 50% methanol  
50% 5.0 mM dodecyltriethyl ammonium  
phosphate with 5.25 mM KH<sub>2</sub>PO<sub>4</sub>  
adjusted to pH 6.2 with NaOH

Injection volume: 200 µL

Wavelength: 217 nm

Chart: 10 mV full scale

Attenuation: 0.01 AUFS

Response time: 5 seconds rise time

Run time: ca. 35 minutes

## F. Analytical Procedure

## 1. Extraction

Weigh 50.0 grams of soil into a 16 oz French square bottle. Add 250 mL of 20% water in acetonitrile. Cap samples and place on a mechanical shaker for 20 minutes. Remove from shaker and filter with suction through a Buchner funnel equipped with a glass fiber filter paper into a 500 mL roundbottom flask. The diameter of the Buchner funnel should be adjusted for the clay content of the soil sample. Samples with high clay content will require a large diameter Buchner funnels. Evaporate the samples to dryness on a rotary evaporator.

## 2. Preparation of Samples for Reverse Phase HPLC

The mobile phase for the reverse phase HPLC is prepared by placing 4000 mL of HPLC grade water in a beaker and adding 22.73 mL of 88% formate buffer to the water. A calibrated pH electrode is placed in the solution, a stir bar is added, and a vortex mixer is placed under the beaker. The pH of the solution is adjusted to 3.8 with gradual addition of ammonium hydroxide while the solution is stirring. This will require approximately 20 mL of ammonium hydroxide. Remove 920 mL of the buffer solution, label, and set aside. Replace the 920 mL of buffer with 920 mL of acetonitrile and continue stirring. After the solution has been thoroughly mixed, filter through a sintered glass filter equipped with a nylon 66 0.45 micron filter into a 4000 mL filter flask.

Rinse HPLC out with filtered HPLC water before placing the mobile phase in the reservoir. It is imperative that the chromatograph be thoroughly flushed with water between each run and stored in acetonitrile:water (50:50). Problems will be encountered with buffer build up on the check valves if a daily maintenance schedule is not incorporated into routine analyses. Also flushing the column with 50:50 acetonitrile:water will remove contaminants from the column and will reduce backpressure problems. Flushing the column with acetonitrile and water should be a standard practice after each analysis.



When the daily startup procedure has been completed, add the degassed filtered mobile phase to the reservoir and begin column equilibration. Place an aliquot of the 920 mL solution containing buffer only (no acetonitrile) in the autosampler reservoir, prime, and wash the autosampler syringe assembly with the new buffer to insure that any residual buffer from the last run has been eliminated from the system.

The remaining buffer solution that contains no acetonitrile is used to dilute to contents of the roundbottom flasks containing the dried soil extract. Each sample is diluted to exactly 5.00 mL with the buffer solution, swirled gently, and filtered through a 3.0 mL disposable syringe with a 0.45 micron luer-lok filter into a autosampler vial. The sample is now ready for HPLC cleanup.

### 3. HPLC Cleanup

The incorporation of an HPLC procedure for the separation of the three ionic metabolites provides a convenient automated approach by which the metabolites can be isolated for subsequent determination by ion pairing HPLC with UV detection. The HPLC system described here consists of an automated sample injector, an HPLC pump, a C8 semi-preparative bonded phase HPLC column, a UV detector, and an automated microprocessor controlled fraction collector. Due to the relatively large sample size required a weaker solvent, the 0.54 N ammonium formate buffer solution, is initially used to preconcentrate the sample near of at the head of the column. This prevents band broadening and allows for smaller fractions to be collected on the fraction collector.

To establish the retention times of I, II, and III, repeat 1000  $\mu$ L injections of a 10  $\mu$ g/mL standard are made. The standard is prepared by taking 1.0 mL of each of the 1000  $\mu$ g/mL solutions of I, II, and III and diluting to 100 mL in a volumetric flask with another aliquot of the freshly prepared buffer solution that contains no acetonitrile. At least three standard injections should be performed to establish the retention time for all three metabolites. The solvent reservoir should be continuously degassed with a small amount of helium during each run. The

reservoir should also be capped to insure that the air above the mobile phase is saturated with formic acid and ammonium hydroxide. If the headspace is not saturated, the effective ionic strength of the mobile phase will decrease with time and the retention time of all three components will increase.

Collection windows for the metabolites are calculated as follows. The order of elution of the three metabolites is II, III, and I respectively. Units for the equations below are in minutes. Note that the connecting tubing between the detector and the fraction collector should be as short as possible. Otherwise the residence time between the detector and the fraction collector will need to be considered when calculating the collection window.

Collection of Metabolite II:

Drain 1: Start of Peak 1 = A  
 Collect 1: {End of Peak 1 - Start of Peak 1} +  
 0.033 {No. of Samples per Run} = B  
 Drain 2: {Start of Peak 2} - {A + B} = C

Collection of Metabolite III:

Collect 2: {End of Peak 2 - Start of Peak 2} +  
 0.033 {No. of Samples per Run} = D  
 Drain 3: {Start of Peak 3} - {A + B + C} = E

Collection of Metabolite I:

Collect 3: {End of Peak 3 - Start of Peak 3} +  
 0.033 {No. of Samples per Run} = F

Occasionally the peaks will elute such that the second term in the Collect (i) will overlap with the start of the following peak. If this occurs, the second term in the equation for the preceding peak should be reduced to the maximum value such that the drain time between the two peaks is zero and the cumulative values of the collection and drain times equals the start time for the ensuing peak. For instance, if the value for drain 3 is a negative number, then the absolute value of E should be subtracted from D and Drain 3 should equal zero. The equation for peak 3 will remain unaffected.

#### 4 Solvent Exchange

Samples from the reverse phase HPLC can either be composited or analyzed individually. If the samples are to be composited, the three metabolite fractions from the HPLC should be quantitatively transferred to a 100 mL roundbottom flask with two 3 mL rinses of 50:50 methanol:distilled deionized water. The samples are taken to near dryness on a rotary evaporator with a 40°C water bath. Successive 5 mL water rinses are added to the flask until the buffer is essentially volatilized. A very minute amount of salt should remain in the flask when the sample is taken to complete dryness. An excessive amount of residual buffer from the reverse phase cleanup in the sample will produce interferences when the samples are quantified on the ion pairing HPLC.

The dried extracts are prepared for the ion pairing HPLC by dilution with freshly prepared ion pairing reagent to a final volume of 20.0 mL using a volumetric pipet. The flasks are swirled gently and filtered through a 3 mL disposable syringe with a 0.45 micron luer-lok Nylon 66 filter. Substitution of a different vendor with respect to the Nylon 66 filter other than the one suggested will likely contribute to interferences from contaminants within the filter assembly. The filtered samples are placed in autosampler vials and are now ready for analysis.

#### 5. Ion Pairing HPLC Analysis

Mobile phase for the ion pairing HPLC is prepared by placing 2000 mL of deionized distilled HPLC grade water in a beaker and adding 1.43 grams of HPLC grade  $\text{KH}_2\text{PO}_4$  to the water. A calibrated pH electrode is placed in the solution, a stir bar is added, and a vortex mixer is placed under the beaker. The pH of the solution is adjusted to 6.2 with gradual dropwise addition of 5% sodium hydroxide while the solution is stirring. The electrode is removed from the solution and two 10 mL bottles of 0.5 M dodecyl triethyl ammonium phosphate ion pairing reagent are added. An additional 2000 mL of methanol is added to the solution and the beaker is stirred until solution is clear and free of bubbles. The mobile phase is filtered through a sintered glass filter equipped with a Nylon 66 0.45 micron filter into a 4000 mL filter flask.

The instrument devoted to the ion pairing HPLC analysis will require careful maintenance in order for it to function properly. Daily rinsing with filtered deionized distilled water at 35°C through the pump and detector is necessary to insure that buffer does not accumulate in the pump and detector cell. A bypass line from the pump directly to the detector should be incorporated into the system to expedite this process. The column should always remain in the ion pairing reagent at >40°C so that buffer does not precipitate out of solution and contribute to an increase in backpressure on the column. The equilibrium in the column should never be disrupted by rinsing with water or methanol. If water or methanol is rinsed through the entire system, including the column, problems will be experienced with contaminants in the flow cell.

Due to the relatively high pH required for the separation, the column will degrade as a function of time. Flow rate and column temperature can be modified to compensate for the deterioration in column efficiency. New columns will require a flow rate of 1.3 mL/minute and a column temperature of 50°C. As the column degrades, flow rate can be gradually reduced to 1.0 mL/minute and the column temperature to 40°C. The column should be replaced when retention times decrease to the point where peak overlap begins to occur. Normal column life is approximately 300-350 injections.

#### 6. Quantitation

The amount of I, II, and III in a given sample is calculated from the equation resulting from the linear least squares analysis of the calibration data. (Calibration data is obtained by plotting  $\mu\text{g}$  of I, II, and III versus peak height) The response of any given sample must not exceed the most concentrated standard. If this occurs, dilution of the sample will be necessary.

#### 7. Interferences

##### 7.1. Sample Matrixes

The analytical method described here has been used for the determination of I, II, and III on seven different soil types. A detailed

description of the soil classifications can be found in Appendix J. No significant interferences were ever observed.

## 2. Other Pesticides

A detailed interference study has not been performed. Field plots treated with Ramrod were also treated with Atrazine 4L, Aatrex 9-0, Roundup, Eradicane, and Milogard. No interferences were noted from any of these other pesticides.

## 3. Solvents

No interferences were observed from pesticide and HPLC grade solvents used for the analyses.

## 4. Labware

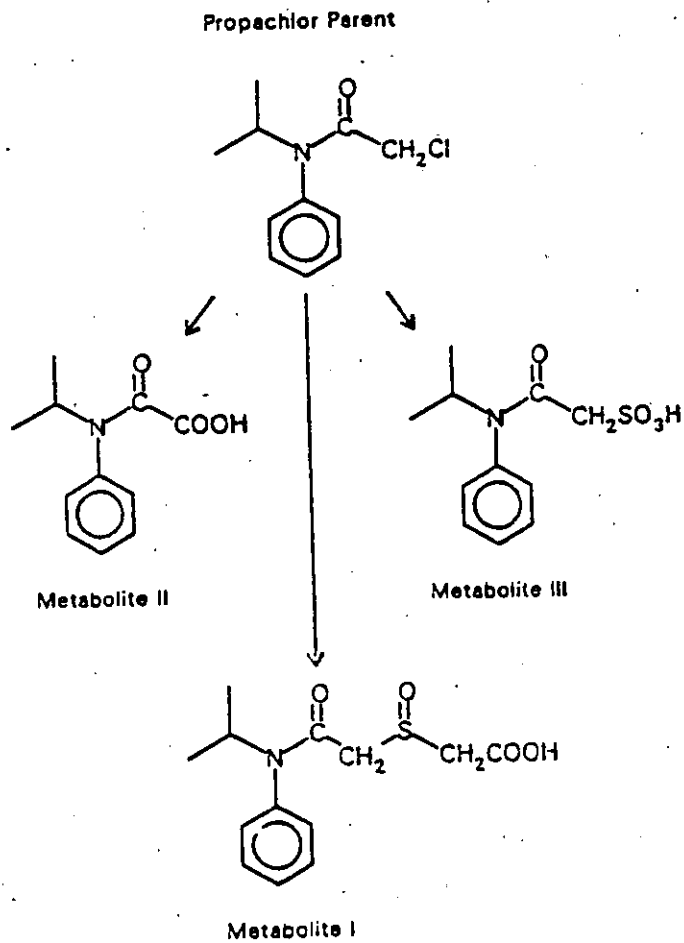
No interferences were noted from glassware used in these analyses. Labware used for handling high levels of I, II, and III (>100 µg/mL) are always discarded after use.

## E. Confirmatory Techniques

A confirmatory technique for the determination of the three ionic metabolites in soil has not been developed.

## F. Time Required for Analysis

A sample set of eighteen samples requires approximately 48 hours from initial extraction to final determination. A portion of this time (approximately 14 hours) is devoted to HPLC cleanup which can be left unattended overnight.

Dissipation of Propachlor  
Into metabolites I, II, and III

Appendix G: Figure 2

PROCEDURE FOR THE ANALYSIS OF  
METABOLITES I, II, AND III

