I. SUMMARY/INTRODUCTION

A. Scope

The analytical procedure described here is for the determination of acetochlor (parent only) in soil treated with acetochlor EC or ME Formulation. The sections in this document list the equipment and chemicals used and describe the preparation of standard solutions and procedures for analyzing acetochlor (parent only) in soil treated with acetochlor EC or ME formulation. This document also provides typical instrument conditions, chromatograms, and validation data for these analyses.

B. Principles

The method consists of extracting a soil sample treated with EC or ME using a two phase solvent system, and after filtering through disposable filtration column, the organic phase is injected directly into a gas chromatograph equipped with a ^{63}Ni electron capture detector for quantitation.

The accuracy of the analytical method is estimated based upon the recovery of known concentrations of acetochlor spiked onto untreated soil, which is then carried through the analytical procedure. The detectability of the method is limited by the presence of background and interferences in the soil. The Limit of Method Validation (LMV) is based upon the acceptable recovery of 0.10 µg of acetochlor which has been fortified onto a 20 g soil sample, or 0.005 ppm.

II. MATERIALS AND METHODS

The following materials, equipment, and reagents are required to perform the analysis. Appropriate substitution for certain items is left to the discretion of the analyst. Cleaning of the glassware and other equipment should be carried out so as to minimize contamination of future samples. The cleaning procedure should be checked to verify appropriate cleanliness. Analysis of reagents and solvents should be carried out to assure a minimum contribution of interferences to actual samples.

A. Equipment

Mettler balance, Model PC 440 and AE 163 or equivalent

120 cc round amber glass bottles and Polyethylene-lined caps Northwestern Bottle Co.

Volumetric pipet, 50 mL: Fisher No. 13-649-P

Volumetric pipet, 100 mL: Fisher No. 13-649-Q

10 mL graduated centrifuge tubes: Fisher No. 05-538-38A 5 mL graduated centrifuge tubes: Fisher No. 05-538-35A

Pasteur pipette 5 3/4 inch length: Fisher No., 13-678-6A

Serological pipettes from 0.10 through 10.0 mL: Fisher No. 13-644 A,B,C,D,E,F,G

Microliter Syringes: Hamilton 10-100 µL

100 mL volumetric flask: Fisher No. 10-210-C

Graduated cylinders, Fisher No. 08-549-5G:H:J.

Mechanical shaker: Fisher No. 14-261

1.8 mL Autosampler vials with teflon lined resealable septa and phenolic caps: Varian No. 96-000099-01

Varian model 3600 or 3700 Gas Chromatograph equipped with a ^{63}Ni electron capture detector, Model 8000 autosampler, and strip chart recorder

Strip chart recorder: Fisher No. 5000

Capillary GC column: J&W DB-210, 0.25 micron film thickness, 30 m x 0.25 mm.

B. Reagents and Standards

Acetonitrile (OPTIMA): Fisher No.: A-996-4

2,2,4-Trimethylpentane (iso-Octane): (OPTIMA) Fisher No. 0-301-4

Ethyl Acetate (OPTIMA): Fisher No.: E-196-4

Deionized water from a Milli-Q water purification system (Millipore Co.). This system consists of an activated carbon cartridge for the removal of organics in series with two mixed-bed ion-exchange cartridges for the removal of ionic species.

Sodium sulfate (NA_2SO_4) anhydrous: Fisher No. S-421

Prepare adequate quantities of each of the following reagents. If it is necessary to remake any of these reagents while in the middle of a set of analyses, it may be necessary to recheck the background resulting from the addition of a new reagent.

10% (v/v) acetonitrile/(di)water

706

Using graduated cylinders, add 100 mL of acetonitrile to 900 mL of distilled deionized water and mix thoroughly.

20% (v/v) ethyl acetate/iso-Octane

Using a graduated cylinder, add 200 mL of ethyl acetate to 800 mL of iso-Octane and mix well.

Acetochlor microencapsulated formulation (MON 8422) normally (41.6% active ingredient w/w.)

Analytical acetochlor standard, purity >99%.

C. Preparation of Standards

1. Acetochlor Neat Fortification Standards.

Weigh to four significant figures 0.1000 grams of analytical grade standard into a 100 mL volumetric flask, and dilute to 100 mL with acetonitrile. Mix well to insure complete dissolution. This stock solution contains 1000 µg/mL of acetochlor.

Pipet 10.0 mL of the 1000 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with acetonitrile and mix well. This solution contains 100 µg/mL.

Prepare a 50 µg/mL solution by adding 5.0 mL of the 1000 µg/mL stock solution to a clean 100 mL volumetric flask and diluting to the mark with acetonitrile. This solution contains 50 µg/mL.

Pipet 10.0 mL of the 100 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with acetonitrile and mix well. This solution contains 10.0 µg/mL.

Pipet 10.0 mL of the 50 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with acetonitrile. This solution contains 5.0 µg/mL.

Prepare a 1.0 µg/mL solution by adding 10.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with acetonitrile. This solution contains 1.0 µg/mL.

Pipet 10.0 mL of the 5.0 µg/mL soluti. .nto a 100 mL volumetric flask and dilute to the mark with acetonitrile. This solution contains 0.5 µg/mL.

Store the standards in properly cleaned and labeled amber glass bottles at 2-6°C.

2. Acetochlor ME Fortification Standards

From a well mixed sample of microencapsulated acetochlor formulation (41.6% active ingredient w/w) weigh the following amounts into separate 100 mL volumetric flasks: 0.2404 gram, 0.0240 gram and 0.0024 gram. Dilute the weighed portions to 100 mL with 10% sodium chloride in water. The concentration of these standards are approximately 1.00, 0.10, and 0.01 µg/µL of acetochlor respectively.

The actual concentration of these standards must be determined by quantitation of aliquots against the neat acetochlor calibration standards. The aliquots must be made into a measured volume of ethyl acetate and shaken for 30 minutes prior to quantitation by GC/ECD. After injection, correction must be applied to account for the final total dilution of the standards.

These standards are used for microencapsulated acetochlor fortifications to soil. Fortifications are made in µL quantities. After preparation, the ME fortification standards are stored in 100 mL volumetric flasks at room temperature.

3. External Acetochlor Standards for GC Quantitation

Weigh to four significant figures 0.1000 grams of analytical grade acetochlor standard into a 100 mL volumetric flask, dissolve and dilute to volume with ethyl acetate. Mix well to insure complete dissolution. This concentrated solution contains 1000 µg/mL of acetochlor.

Pipet 10.0 mL of the 1000 µg/mL solution into a 100 mL volumetric flask and dilute with 20% ethyl acetate/iso-Octane to the mark and mix well. This solution contains 100 µg/mL.

Pipet 10.0 mL of the 100 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with 20% ETOAC/I.O. This solution contains 10.0 µg/mL.

Prepare a 1.0 µg/mL solution by adding 10.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask and dilute to the mark with 20% ETOAC/I.O. This solution contains 1.0 µg/mL.

Pipet 10.0 mL of the 1.0 µg/mL solution into a 100 mL volumetric flask and dilute with 20% ETOAC/I.O. to the mark.

This solution contains 0.10 µg/mL.

Prepare separately the GC calibration standards in 100 mL volumetric flasks according to the following scheme.

	Volume of the 0.10 µg/mL Standard	Dilution with 20% ETOAC/I.O.	Final Concentration Acetochlor µg/mL
STD-1	0.50 mL	100.0 mL	0.00050
STD-2	1.00 mL	100.0 mL	0.00100
STD-3	2.00 mL	100.0 mL	0.00200
STD-4	5.00 mL	100.0 mL	0.00500
STD-5	7.00 mL	100.0 mL	0.00700
STD-6	10.00 mL	100.0 mL	0.01000
	Volume of the 1.0 µg/mL Standard		
STD-7	1.20 mL	100.0 mL	0.01200
STD-8	1.40 mL	100.0 mL	0.01400
STD-9	2.00 mL	100.0 mL	0.02000

Standard solutions are stored in properly cleaned and labeled amber glass bottles at 2-6°C. New detector calibration standards should be prepared from the stock solution approximately every 6 months. Extreme care should be taken to prevent cross contamination of the lower standards with the higher concentration standards.

D. Analytical Procedure

A 20 g subsample of a homogeneous mixture of the soil being analyzed is solvent extracted to remove acetochlor for direct separation and quantitation by gas chromatography.

1. Sample Preparation

Soil samples are taken in the field with a soil probe which uses a plastic liner to hold the soil core in place. The soil sample in the plastic liner is properly labeled and frozen immediately for shipment to the Residue section at Monsanto. The frozen soil core is then cut into the appropriate depth segments. Multiple samples from each sampling are combined and mixed

thoroughly to obtain a homogenous sample. After mixing, the samples are stored frozen until analyzed.

2. Extraction and Fortification

Weigh 20 g (± 2%) of the previously prepared soil into a 120 cc glass bottle (fortify at this step)*. Add 10 mL of 10% acetonitrile/water to the bottle and 50 mL 20% ethyl acetate/iso-Octane, cap tightly and shake on a linear reciprocating shaker for 1 hour. After shaking, allow the sample to settle for at least one hour or over night.

3. Filtration

Due to the possibility of introducing extremely fine soil particles upon injecting the sample extract into the flash injector, the sample must be filtered prior to injection. However, we have found that most commercially available filters, even those claiming compatibility with organic solvents, introduce contaminants into the sample which can interfere with the quantitation of acetochlor. It has been found that a glass pasteur pipette plugged with a small amount of glass wool, topped with 2-3 cm of anhydrous sodium sulfate (for removal of residue water) works adequately for the removal of any soil particles which remain suspended in the organic phase of the extract.

To filter the sample, prepare a pasteur pipette as described previously and rinse the column to remove any contaminant by allowing at least 1-2 mL of the organic extract to pass through the column before collecting the sample to be used for GC quantitation. NOTE: DO NOT rinse the column with any other solvent prior to filtering the extract. This will dilute the sample. Rinse the column only with the sample extract prior to collecting the final aliquot. The sample is now ready for GC separation and subsequent quantitation.

* Example: Pipet 0.2 mL of the 0.5 µg/mL of the Acetochlor standard in acetonitrile directly on the check sample matrix for a 0.005 ppm fortification. For the microencapsulated acetochlor fortifications, syringe appropriate microliters of encapsulated acetochlor standard under continuous agitation directly onto the check soil in the bottle.

III. INSTRUMENTATION

1. Gas Chromatograph

A Varian gas chromatograph equipped with a ^{63}Ni electron capture detector has been used to separate and quantitate acetochlor in the soil extract.

GC Column:

J &W Scientific Fused silica capillary column
Stationary phase: DB-210
Film Thickness: 0.25 micron
Column Dimensions: 30 m x 0.25 mm

2. Operating Conditions

Operate the gas chromatograph using the following conditions. Be aware that different instruments may require modification of these parameters in order to achieve satisfactory sensitivity and separation of acetochlor from co-extracted species. Follow the procedure recommended by the manufacturer of the instrument regarding operation and optimization.

Column Temperature	150°C hold for 1 min then program at 3°C/min to 240°C and hold for 5 min
Injector Temperature	250°C
Detector Temperature	300°C
Injection Volume	5 µL
Detector Attenuation	16
Detector Range	1
Carrier Gas (Nitrogen)	2 mL/min
Detector Makeup (Nitrogen)	28 mL/min
Split Flow	4 mL/min
Split Ratio	2:1
Quantification	External standards, Peak height

3. Detector Calibration

A linear calibration curve is generated for every set of samples run. Several levels of standards are prepared in the range of 0.0005 to 0.02 µg/mL. These standards are periodically placed among the analytical samples.

The calibration curve is generated by plotting the peak height of the detector response against the concentration of each calibration standard of acetochlor.

The response of any given sample must not exceed the response of the most concentrated standard. If this occurs, dilution of the sample will be necessary with 20% ethyl acetate/iso-Octane.

IV. SOIL MOISTURE DETERMINATION

The calculation of the concentration of acetochlor is made with respect to the dry soil mass analyzed; (except recoveries) therefore, the percent moisture of each sample must be determined.

A. Procedure

Weigh a glass container, such as a 60 or 100 mL beaker, and record this weight to a hundredth of a gram. Next, weigh out an aliquot of 20.0 g ± 10% of the soil sample analyzed, record this weight to a hundredth of a gram. Total the weight of the container plus the weight of the soil and record this weight. Place the container containing the soil in a dry heat oven set to at least 120°C for at least 12 hours. After this period, remove the container and allow to cool. Reweigh the container plus the dry soil and record this weight. The amount of moisture contained in that soil is the difference between the combined (container + soil) weight before drying and the combined weight after drying. The percent soil moisture is determined by dividing the amount of soil moisture by the weight of wet soil before drying times 100. This calculation is illustrated in the following section.

V. CALCULATIONS

Calculations of amounts of acetochlor found in each analytical sample, the estimated accuracy for the set, the percent soil moisture, and the ppm concentration of acetochlor are calculated as described.

A. Quantitation of Acetochlor

The concentration of acetochlor in the soil extract is determined based upon the peak height of the elution peak of acetochlor. The

concentration is determined by comparison of peak heights to a calibration curve generated linearly from concurrently run external standards.

Due to limited linear range of the electron capture detector, proper dilution of each sample must be made to keep the peak height response of acetochlor within the limits of the calibration standards. Since the LMV is obtained in the original 50 mL extract, this is the most concentrated sample. If a sample is found to contain a concentration of acetochlor greater than the highest calibration standard, it must be diluted accordingly with 20% ethyl acetate/iso-Octane. This dilution must be recorded in order to obtain the total amount of acetochlor found in the soil extract for PPM calculation.

Percent recovery is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{PPM Found}}{\text{PPM Fortified}} \times 100$$

If the check sample matrix was found to contain acetochlor, then this amount must be subtracted from the amount found in the spiked sample in order to calculate recovery for that sample.

B. Percent Moisture

The percent soil moisture is determined for a given sample in order to calculate acetochlor residues based on dry soil weight.

$$\frac{\text{(Combined wet wt - Combined dry wt)}}{\text{Wet soil wt}} \times 100 = \% \text{ soil Moisture}$$

C. Acetochlor Residues

After determining the concentration of acetochlor in the sample extract, the total amount of acetochlor residue in the 20 g sample (ppm) is determined. To do this, the concentration (µg/mL) of acetochlor in the extract is multiplied by the total dilution volume of the extract to obtain the total amount (µg) of acetochlor in the soil sample analyzed. The total amount of acetochlor is divided by the dry soil weight (grams) of the sample analyzed resulting in µg/gram or parts per million (ppm). This procedure is illustrated below.

$$\frac{\text{µg acetochlor}}{\text{mL}} \times \frac{\text{dilution volume}}{\text{dry soil weight}} = \text{ppm Acetochlor}$$

where

$\frac{\mu g \text{ acetochlor}}{mL}$ is the concentration of Acetochlor in the extract

Dil Vol is the total dilution volume (mL) of the extract taking into account any further dilution necessary to maintain the peak height of the analyte within the highest standard of the calibration curve.

Wet Soil Wt is the weight (g) of the soil sample analyzed

% Soil Moisture is the amount, expressed as a percent, of moisture in the soil analyzed.

ppm is the concentration, expressed as parts per million, of acetochlor residue in dry soil.

VI. INTERFERENCES

1. Soil Sample

   Using the analytical method described here, very little or no background has been observed. The background concentration is subtracted from fortified samples prior to calculating recovery.

2. Solvent

   No interferences have been observed when using high purity solvents and reagents.

3. Labware

   The glassware cleaning procedure consists of washing the glassware in a mechanical washer with hot soapy water followed by deionized water rinse and final acetone rinse. No interferences have been observed.

VII. TIME REQUIRED FOR ANALYSIS

A set of 10 samples requires approximately 8 hours from initial extraction to GC-ECD quantitation.

VIII. MODIFICATION OR POTENTIAL PROBLEMS

Variations in reagents or in manufacturers may result in interferences.

IX. METHOD OF CALCULATION

The amount of acetochlor is determined based upon standard calibration. A non-weighted linear least squares fit of the calibration curve is used to calculate the amount of acetochlor in the unknown samples. The response

of any given sample must not exceed the response of the most concentrated standard. If this occurs, dilution of the sample will be necessary and sample should be re-analyzed.

X. LIMIT OF QUANTIFICATION (LOQ)

The lowest fortification level that was actually used in validation studies for this method was 0.005 ppm. The average recovery obtained for samples at this level (and level tested) during method validation was judged acceptable (i.e., average recovery exceeded 70%). For this reason, the reporting limit for results obtained in the present study is set at this lower method validation limit and all measured concentrations below 0.005 ppm are reported merely as "<0.005 ppm".

XI. EXAMPLE CHROMATOGRAMS

Example chromatograms are attached for the analysis of acetochlor in soil.

ANALYTICAL METHOD FOR THE DETERMINATION OF THE THREE ACETOCHLOR METABOLITES IN SOIL

I. SCOPE

The analytical method described here is for the determination of the three major acetochlor metabolites in soil: 1. Acetic acid, [(ethoxymethyl)(2-ethyl-6-methylphenyl)amino]oxo-,sodium salt, 2. Acetic acid,{[2-[(ethoxymethyl)(2-ethyl-6-methylphenyl)amino]-2-oxoethyl]sulfinyl}-,sodium salt, 3. Ethanesulfonic acid, 2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)amino]-2-oxo-,sodium salt. The sections in this document list the equipment and chemicals used and describe the preparation of standard solutions for analyzing these three acetochlor metabolites in soil. This document also provides typical instrument conditions, chromatograms and validation data for this analytical method.

II. PRINCIPLES

The analytical method described determines residues of acetochlor metabolites in soil. The procedure consists of extraction of a soil sample with 60% acetonitrile-water followed by high speed centrifugation. The supernatant liquid is passed through an amine solid phase cartridge which contains a pre-column solid phase bed of Florisil/C^{18}/activated carbon. The metabolite solution is collected, evaporated to dryness, and the residue taken up in a 15% acetonitrile-potassium phosphate buffer. Quantitation is by HPLC using a C^{18} analytical column with UV detection at 210 nm. The method has been validated to 0.010 ppm of all three acetochlor metabolites in soil. This is based upon the acceptable recovery of 0.5 µg of the three metabolites which has been fortified onto a 50 gm soil sample.

III. MATERIALS AND METHODS

A. EQUIPMENT

The following materials, equipment and reagents are required to perform the analysis. Appropriate substitution for certain items is left to the discretion of the analyst. Cleaning of the glassware and other equipment should be carried out so as to minimize contamination of future samples. The cleaning procedure should be checked to verify appropriate cleanliness. Analysis of reagents and solvents should be carried out to assure a minimum contribution of interferences to actual samples.

250 mL Polypropylene Bottles and seal caps, DuPont Company, No. 03937

Mettler balance, Model PC-440 or equivalent

Two speed shaker- Eberback 6010: American Scientific Products No. S1105

Vacuum Manifold 24-port: American Scientific Products No. 9401

Sorvall RC2B or RC5 Superspeed Automatic Refrigerated centrifuge. Sorvall Instruments, Wilmington, DE or equivalent

Calab rotary evaporator

Hot plate with water bath

500 mL Round Bottom Flask: Fisher No. 10-067G

100 mL Round Bottom Flask

5 mL Graduated centrifuge tubes: Fisher No. 10-437-10C

Pasteur Pipette 5 3/4" length: Fisher No. 13-678-6A

Fisher Accumet Model 815MP pH Meter

Perkin-Elmer ISS-100 automatic sampler equipped with a 100 µL injection loop: Perkin Elmer No. 0254-0655

DuPont Zorbax ODS, 0.46 x 25 cm analytical Column, No. 880952-702 Available from MAC-MOD Analytical, Chadds Ford, PA

Zorbax ODS Guard Column, 0.40 x 1.25 cm, No. 820674-902

Zorbax Reliance Guard Column Holder, No. 820529-901

Fisher Recordall Series 5000 strip chart recorder

Vials for HPLC autosampler: Varian No. 66-000104-00

Four column HPLC Temperature Controller: Rainin Instrument Co. No: ELD=1233

Nylon 0.22 micron membrane filters, Fisher No. NO2SP040700

MSI Filter Unit, Teflon, 0.2 Micron, Cat. No. DDF02T2550

30 mL disposable syringes, Fisher No. 14-823-2C

Assorted standard laboratory equipment

B. REAGENTS AND STANDARDS

723

The following reagents are used in this analytical method. Specific brands are listed to aid the chemist in obtaining these items. In most cases equivalent reagents can be used which can obtained from other vendors.

Acetonitrile (ACN), Fisher No. A-998

Deionized water from a Milli-Q water Purification system (Millipore Co). This system consists of an activated carbon cartridge for the removal of organics in series with two mixed-bed ion exchange cartridges for the removal of ionic species.

50% Acetonitrile in water and 60% Acetonitrile in water

Decolorizing Carbon, Aldrich Chemical Co. No. 24,227-6

Florisil, 60-100 mesh, Fisher No. F100-500

C^{18} bonded phase column packing, VWR Scientific No. JT7025-1

C^{18}:Florisil:Carbon Mixture: Weigh 331 g of C^{18}, 164.5 g of Florisil 4.15 g Decolorizing Carbon into clean dry amber bottle and place on shaker for 1 hour, turning bottle after 1/2 hour

NH_2 bonded phase columns, 60 cc, Analytichem International No. 1225-6036

Potassium Hydroxide Pellets, Fisher No. P250-500

KOH solution, 10 gm of KOH pellets in 100 mL Distilled Water

8.5% Phosphoric Acid solution, 10 mL of 85% H_3PO_4 diluted to 100 mL with Distilled Water

Potassium Phosphate Monobasic, Mallinckrodt No. 7100

Mobile phase B:

50% Acetonitrile/buffer solution: Dissolve 10.88 g of potassium phosphate in 2000 mL of HPLC grade deionized water, add 2000 mL of acetonitrile and adjust to pH 6 using KOH solution. Vacuum filter through a 0.22 micron Nylon filter membrane.

Mobile phase A:

15% Acetonitrile/buffer solution: Dissolve 10.88 g of potassium phosphate in 3400 mL of HPLC grade deionized water, add 600 mL of

acetonitrile and adjust to pH 6 using KOH solution. Vacuum filter through 0.22 µm Nylon filter membrane. 24

Acetochlor metabolites all of minimum acceptable purity, 90%, if obtainable. If purity values less than 90% are used, appropriate corrections should be made to standard concentrations.

1. Acetic acid, [(ethoxymethyl)(2-ethyl-6-methylphenyl)-amino]oxo-,sodium salt. CP95200 - Common name : Oxanilic acid
2. Acetic acid, [(2-[(ethoxymethyl)(2-ethyl-6-methylphenyl)-amino]-2-oxoethyl)sulfinyl]-,sodium salt. CP97290 - Common name : Sulfinyl acid
3. Ethanesulfonic acid, 2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)-amino]-2-oxo-,sodium salt. CP92429 - Common name : Sulfonic acid

C. PREPARATION OF STANDARDS

1. Fortification Standards

Weigh to four significant figures 0.10 gram of analytical grade CP 95200, CP 92429, and CP 97290 into a 100 mL volumetric flask. Dilute to volume with 50% ACN/water and mix well to insure complete dissolution. This solution contains 1000 µg/mL of all three metabolites.

Pipette 10.0 mL of the 1000 µg/mL solution into a 100 mL volumetric flask, dilute to volume with 50% water/acetonitrile and mix well. This solution contains 100 µg/mL of all three metabolites.

Pipette 10.0 mL of the 100 µg/mL solution into a 100 mL volumetric flask, dilute to volume with 50% water/acetonitrile and mix well. This solution contains 10 µg/mL of all three metabolites.

Pipette 10.0 mL of the 10 µg/mL solution into a 100 mL volumetric flask, dilute to volume with 50% water/acetonitrile and mix well. This solution contains 1.0 µg/mL of all three metabolites.

Fortification solutions are stored refrigerated in properly cleaned and labeled amber bottles.

2. HPLC-UV Quantitation Standards

Pipette 10.0 mL of the 1000 µg/mL solution prepared above into a 100 mL volumetric flask, dilute to volume with 15%

acetonitrile/buffer and mix well. This solution contains 100 µg/mL of all three metabolites.

Pipette 10.0 mL of the 100 µg/mL solution into a 100 mL volumetric flask, dilute to volume with 15% acetonitrile/buffer and mix well. This solution contains 10 µg/mL of all three metabolites.

Prepare the HPLC calibration standards in 100 mL volumetric flasks and dilute to volume according to the following scheme:

Volume of 10.0 µg/ml Standard	Standard Dilution	Final Concentration
50.0 mL	100.0	5.000 µg/mL
25.0 mL	100.0	2.500 µg/mL
10.0 mL	100.0	1.000 µg/mL
5.0 mL	100.0	0.500 µg/mL
2.5 mL	100.0	0.250 µg/mL
1.0 mL	100.0	0.100 µg/mL

Each of the calibration standards is diluted to the 100 mL final volume with 15% acetonitrile/buffer.

Standard solutions are stored in properly cleaned and labeled amber glass bottles and stored refrigerated.

D. ANALYTICAL PROCEDURE

A 50 g subsample of a homogeneous mixture of the soil being analyzed is solvent extracted to remove all three acetochlor metabolites for clean-up, separation and quantitation by HPLC.

1. MOISTURE DETERMINATION

Pre-mixed soil samples (20.0 ± 0.05 g) are weighed into a tared beaker. The soil is placed in a oven at approximately 130°C for a minimum of 12 hours. The dried soil sample is weighed again, the weight loss being attributed to the moisture content of the soil.

2. RESIDUE DETERMINATION

Soil samples are taken in the field with a soil probe which uses a plastic liner to hold the soil core in place. The soil sample in the plastic liner is properly labeled and frozen immediately for shipment to the Residue section at Monsanto. The frozen soil core is then cut into the appropriate depth segments. Multiple samples for each sampling are combined and mixed

thoroughly to obtain a homogeneous sample. After mixing, the samples are stored frozen until analyzed.

3. EXTRACTION

Weigh 50.0 ± 0.04 g of soil into a 250 mL centrifuge bottle. Fortify at this stage. Example: Pipette 0.5 mL of the 1.0 µg/mL metabolite fortification solution directly onto the soil for a 0.01 ppm fortification of each species. Add 200 mL of 60% ACN/water solution to the bottle and shake for thirty minutes. Centrifuge for 20 minutes at 10,000 RPM at a temperature between -5° to +5° C. If the sample appears to have a significant amount of suspended solids or a muddy appearance after the first centrifugation, decant the sample into another centrifuge bottle, add 10 g of Florisil, and recentrifuge.

4. COLUMN CLEAN-UP

Place NH_2 column on vacuum manifold and add a 10 g scoop of the C^{18}:Florisil:Carbon mixture. Prewash the column with 25 mL of water followed by 50 mL of the 50% ACN/water solution. Transfer the sample from the centrifuge bottle to the column, collecting the eluant in a 250 mL centrifuge bottle. Wash the column with 50 mL of 50% ACN/water. If the eluant has suspended particulates, centrifuge for 10 minutes at 10,000 RPM or filter through glass wool. Transfer to a 500 mL round bottom flask. Evaporate to approximately 1 mL using a rotary thin-film evaporator with the flask immersed in a 40°C water bath. (Note: the water bath should be room temperature at first and the temperature should be raised to 40°C after vacuum is applied to the flask).

Add 30 mL of acetonitrile and three to five drops of the 8.5% phosphoric acid solution and mix well by swirling the round bottom. Allow this solution to sit with periodic swirling for approximately 2-3 minutes. At this stage a precipitate may be present. Quantitatively transfer the sample to a 30 mL disposable syringe fitted with a MSI 0.22 micron disk filter. Filter this solution directly into a clean, dry 100 mL round bottom flask. This resulting solution is evaporated to dryness on the rotary thin-film evaporator with the flask immersed in room temperature water. Quantitatively transfer the residue sample to a 5 mL graduated centrifuge tube using 2 x 1.5 mL of the 15% ACN/buffer mobile phase solution for a total of 3.0 mL. This solution should be mixed well and filtered through a MSI 0.22 micron filter unit before injection onto the HPLC for separation and Quantitation.

IV. INSTRUMENT PARAMETERS

A Perkin Elmer HPLC equipped with UV detector has been used to separate and quantitate the three acetochlor metabolites in the soil extract.

A. OPERATING CONDITIONS

Operate the HPLC using the following conditions. Be aware that different instruments may require modification of these parameters in order to achieve satisfactory sensitivity and separation of the three acetochlor metabolites from co-extracted species. Follow the procedure recommended by the manufacturer of the instrument regarding operation and optimization.

Instrumentation:	Perkin Elmer Series 4 Liquid Chromatogram Perkin Elmer LC-95 UV/Visible Spectrophotometer Detector
or:	
	Varian 9010 Ternary Gradient Pump Varian 9050 UV-Visible Detector

HPLC Conditions:

Column:	DuPont Zorbax ODS, 0.46 x 25 cm. Zorbax ODS Guard Column, 0.40 x 1.25 cm.
Temperature:	55°C
Detection:	UV detector at 210 nm
Flow rate:	0.9 mL/min
Mobile Phase:	A. 15% acetonitrile/buffer
	B. 50% acetonitrile/buffer
Response time:	500 msec
Sample size:	100 µL

IX. METHOD OF CALCULATION

Calculations of amounts of the three metabolites found in each analytical sample, the estimated accuracy for the set, the percent soil moisture, and the ppm concentration of the acetochlor metabolites are calculated as described.

A. QUANTITATION OF ACETOCHLOR METABOLITES

The concentration of the acetochlor metabolites in the soil extract is determined based upon the peak area of the elution peak of each metabolite. The concentration is determined by comparison of peak areas to a calibration curve generated linear from concurrently run external standards.

Percent recovery is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{PPM Found}}{\text{PPM Fortified}} \times 100\%$$

If the check sample matrix was found to contain one of the metabolites, then this amount must be subtracted from the amount found in the spiked sample in order to calculate recovery for that sample.

B. PERCENT MOISTURE

The percent soil moisture is determined for a given sample in order to calculate the metabolite residues based on dry soil weight.

$$\frac{\text{Combined wet wt.} - \text{Combined dry wt.} \times 100}{\text{Wet soil wt.}} = \% \text{ soil moisture}$$

C. ACETOCHLOR METABOLITE RESIDUES

After determining the concentration of each metabolite in the sample extract, the total amount of metabolite residues in the 50 g sample (ppm) is determined. To do this, the concentration (µg/mL) of the metabolites in the extract is multiplied by the total dilution volume of the extract to obtain the total amount (µg) of each metabolite in the soil sample analyzed. The total amount of each metabolite is divided by the dry soil weight (grams) of the sample analyzed resulting in µg/g or parts per million (ppm). This procedure is illustrated below.

$$\frac{\mu\text{g of Metabolite}}{\text{mL}} \times \frac{\text{dilution volume}}{\text{dry soil wt}} = \text{ppm metabolite}$$

where

$\frac{\mu g \text{ of Metabolite}}{mL}$ = the concentration of metabolite in the extract

Dilution Volume = the total dilution volume (mL) of the extract taking into account any further dilution necessary to maintain the peak height of the analyte within the highest standard of the calibration curve.

Combined Wet Weight = the weight of the wet soil plus the beaker

Combined Dry Weight = the weight of the dry soil plus the beaker

Wet Soil Wt = the weight (g) of the soil sample analyzed

% Soil Moisture = the amount, expressed as a percent, of moisture in the soil analyzed.

ppm is the concentration, expressed as parts per million, of each metabolite residue in dry soil.

X. VALIDATION OF METHOD

During method development, the lowest limit at which the method was validated for each metabolite is 0.01 ppm. An example of method validation data is presented in Figure 2.