ANALYTICAL METHOD FOR THE DETERMINATION OF ACETOCHLOR IN SOIL TREATED WITH MON-8460

1. SCOPE

This analytical method determines residues of acetochlor (MON 097), a chloroacetanilide herbicide, in soil.

2. INTRODUCTION

The analytical method described determines residues of acetochlor (parent) in soil. The method consists of extracting a soil sample using a two phase solvent system, and after filtering, injecting the organic phase 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 are 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 quantitation is based upon the lowest fortification level recovered with acceptable efficiency, i.e., greater than 70%. This method has been validated to 0.005 ppm.

3. DETECTABILITY

The Limit of Method Validation (LMV) of this procedure is 0.005 ppm of acetochlor in soil. The Limit of Method Validation 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.

4. MATERIALS AND REAGENTS

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.

5. MATERIALS AND EQUIPMENT

Mettler balance, Model PE-3600 or equivalent

French square glass bottles, 4 oz.: Northwestern Bottle Co., St. Louis, Mo.

Volumetric pipet, Class A, 25 ml: Fisher No. 13-650-2S

10 mL graduated centrifuge tube: Fisher No. 05-538-38A

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

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

1.8 mL autosampler vials with teflon lined resealable septa and phenolic caps: Varian No. 96-000099-00

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

J & W direct flash injector liner for Megabore® columns: Catalog No. 2101064

J & W DB-17 fused silica Megabore® column, 30 m length, 0.520 mm I.D., 1.50 µm film thickness: Catalog No. 125-1732

Glass wool: Fisher No. 11-390

6. REAGENTS

Acetonitrile: Scientific Products No. 2442-4NY

Absolute ethanol, reagent grade

2,2,4-Trimethylpentane (isooctane): Scientific Products No. 6051-4NY

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

7. REAGENT PREPARATION

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/WATER

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

8. PREPARATION OF ANALYTICAL STANDARDS

A. FORTIFICATION STANDARDS

Weigh 0.1000 gram of analytical grade acetochlor into a 100 mL volumetric flask and dilute to volume with absolute ethanol. This solution contains 1000 µg/mL.

Pipet 10.0 ml of the 1000 µg/ml solution into a 100 mL volumetric flask and dilute to volume with absolute ethanol. This solution contains 100.0 µg/mL.

Dilutions can be made from the 100.0 µg/ml solution as follows:

Acetochlor Concentration Used	Milliliter Used	Standard Dilution	Acetochlor Concentration µg/mL
100 µg/mL	1.0	100.0	1.0
100 µg/mL	2.0	100.0	2.0
100 µg/mL	5.0	100.0	5.0
100 µg/mL	10.0	100.0	10.0
100 µg/mL	25.0	100.0	25.0

Standard solutions are stored in properly cleaned and labeled amber glass bottles at 2-6°C.

B. DETECTOR CALIBRATION STANDARDS

Weigh 0.1000 g of acetochlor into a 100 mL volumetric flask and dilute to volume with isooctane. This solution contains 1000 µg/mL.

Pipet 10.0 ml of the 1000 µg/mL solution into a 100 mL volumetric flask and dilute to volume with isooctane. 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 volume with isooctane. This solution contains 10 µg/mL.

Pipet 10.0 ml of the 10 µg/mL solution into a 100 mL volumetric flask and dilute to volume with isooctane. This solution contains 1 µg/mL.

Pipet 10.0 mL of the 1 µg/mL solution into a 100 mL volumetric flask and dilute to volume with isooctane. This solution contains 0.1 µg/mL.

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

Volume of 0.1 µg/mL Acetochlor standard	Dilution with Isooctane	Final Concentration
1.0 mL	100.0	0.001 µg/mL
2.0 mL	100.0	0.002 µg/mL
5.0 mL	100.0	0.005 µg/mL
7.0 mL	100.0	0.007 µg/mL
10.0 mL	100.0	0.010 µg/mL

Volume of 1.0 µg/ml Acetochlor standard	Dilution with Isooctane	Final Concentration
2.0 mL	100.0	0.020 µg/mL
5.0 mL	100.0	0.050 µg/mL
7.0 mL	100.0	0.070 µg/mL
10.0 mL	100.0	0.010 µg/mL

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 solutions approximately every 6 months. Extreme care should be taken to prevent cross contamination of the lower standards with the higher concentration standards.

9. SAMPLE PREPARATION AND EXTRACTION

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.

A. 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 until analyzed.

B. EXTRACTION

Weigh 20 g (± 0.04) of the previously prepared soil sample into a glass bottle (fortify at this step)*. Add 10 mL of 10% acetonitrile/water to the bottle and 25 mL isooctane, cap tightly and shake on a linear reciprocating shaker for 10 minutes. After shaking, allow the sample to settle for at least 5 minutes.

* Example: Pipet 0.10 of the 1.0 µg/mL acetochlor standard in ethanol directly on the sample matrix for a 0.005 ppm fortification.

C. 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 pastuer pipette plugged with glass wool, topped with 2-3 cm of anhydrous sodium sulfate (for removal of residual 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 pastuer pipette as described previously and rinse the column to remove any contaminants by allowing at least 5 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 isooctane solvent, or 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.

10. SEPARATION AND DETECTION

A Varian model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector has been used to separate and quantitate acetochlor in the soil extract.

A. 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 procedures recommended by the manufacturer of the instrument regarding operation and optimization.

Column: J & W DB-17 fused silica Megabore column, 30 M x 0.52 mm I.D., 1.5 µm film thickness

Column Temperature: 220°C

Injector Temperature: 250°C

Detector Temperature: 330°C

Carrier Gas: Nitrogen at 7.0 mL/min plus 20 mL/min makeup at the detector inlet

Injection Mode: J & W Direct-flash glass injector liner

Injection Volume: 5 µL

Detector Attenuation: 128

Detector range: 1

B. DETECTOR CALIBRATION

A exponential calibration curve is generated for every set of samples run. Nine levels of acetochlor standards are prepared in the range of 0.001 to 0.10 µg/mL. These standards are periodically placed among the analytical samples.

The calibration curves are 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.

11. SOIL MOISTURE DETERMINATION

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

A. PROCEDURE

Weigh a glass container, such as a 100 mL beaker, and record this weight to a hundredth of a gram. Next, weigh out an aliquot of 20.00 g 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.

13. 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 exponentially 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 calibratrion standards. Since the LMV is obtained in the original 25 mL extract, this is the most concentrated a sample must be. If a sample is found to contain a concentration of aceto-chlor greater than the highest calibration standard, it

must be diluted accordingly with isooctane. 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.

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

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. This 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.

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

and

$\frac{\mu g \text{ Acetochlor}}{mL}$ is the concentration of acetochlor in soil 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 acetochlor is the concentration, expressed as parts per million, of acetochlor residue in dry soil