I. SUMMARY/INTRODUCTION

A. SCOPE

This analytical method is for the determination of MON 37500 and its major metabolite in soil. The method employs high performance liquid chromatography (HPLC) to quantitate the analytes extracted from the soil after hydrolysis to the rearrangement product.

B. PRINCIPLES

Metabolism studies have identified the sulfonamide and the aminopyrimidine as the only significant metabolites of MON 37500 in soil. The aminopyrimidine has been well characterized in several other sulfonylurea herbicides, which leaves the sulfonamide as the only metabolite unique to MON 37500. The MON 37500 is hydrolyzed to its rearrangement product and is quantitated by HPLC, along with the sulfonamide, with fluorescence detection. See Figure 1 for a diagram of the reactions.

Conversion of the MON 37500 to its rearrangement product requires base hydrolysis by refluxing at atmospheric pressure for one hour.

The soil sample is extracted twice, first with an acetonitrile/water mixture and then with an acetonitrile/NaOH mixture. After centrifuging and combining the extracts, the sample is made basic with NaOH and refluxed for 1 hour. After reaction is complete, the sample is acidified with HCl and extracted into methylene chloride. The sample is then cleaned up on florisil and concentrated for HPLC analysis. See Figure 2 for a general schematic of the method described.

Accuracy of the analytical method is estimated based upon the recovery of known concentrations of MON 37500 spiked onto untreated soil samples which are then carried through the analytical procedure. Average recovery is determined for each matrix. The lower limit of method validation (LLOMV) for this method is 0.001 ppm for MON 37500 and 0.005 ppm for sulfonamide. This equates to 0.05 µg MON 37500 and 0.25 µg sulfonamide fortified onto a 50.0 gram sample. An average recovery of 70% or better is expected.

MON 37500 in Soil

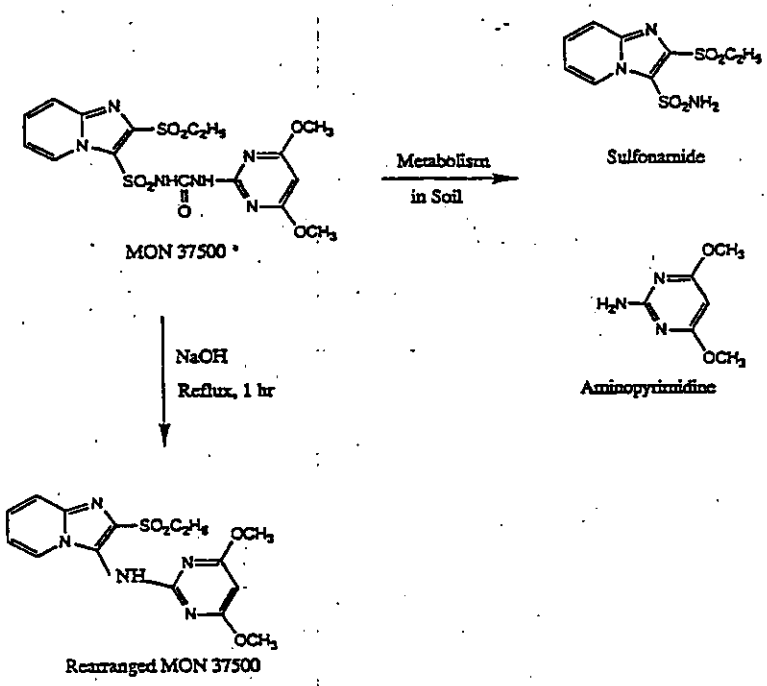


Figure 1

MON 37500 Soil Method

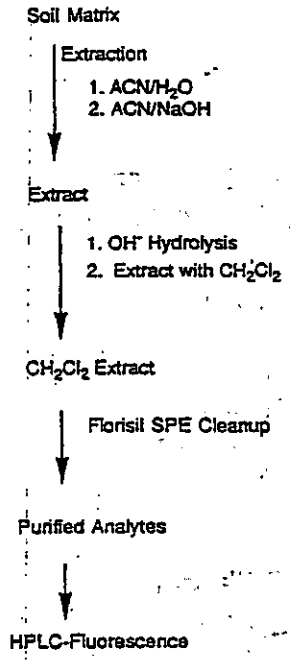


Figure 2

II. MATERIALS/METHODS

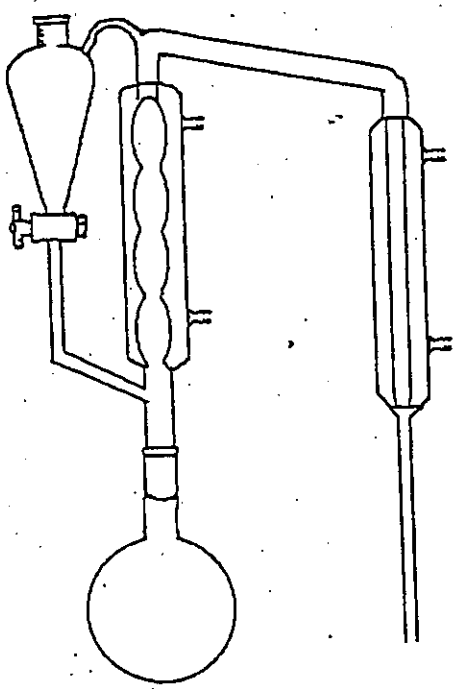
The following materials, equipment, and reagents are required to perform the analysis. Appropriate substitution for certain items is left to the discretion of the analyst unless otherwise noted. Cleaning of the glassware and other equipment should be carried out so as to minimize contamination of future 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

1.) General:

- 100 mL volumetric flask: Fisher No. 10-210 C
- Mettler electronic top-loading balance, model PM4600
- Centrifuge bottles: 250 mL, Fisher No. 05-564
- Centrifuge: Sorvall superspeed RC2-B
- Mechanical shaker
- Funnel, Fisher No. 10-346B
- Separatory funnel, 250 mL with teflon stopper
- Beaker, 200-400 mL
- Round bottom flasks: 500 mL with stoppers
- Brinkman Dispensette, bottle-top dispensers, various sizes: Fisher No. 13-688-71
- Volumetric pipettes, various sizes
- Teflon stoppers S/T 19
- Teflon coated stir bars: Fisher No. 14-511-58A
- pH paper, range 0-6, and 7-14: Scientific Products No. P1119-5A,7A
- Pasteur pipette, 5¾ and 9 inch length: Fisher No. 13-6678-6A,B
- Heating mantle for 500 mL round bottom flask: Fisher No. 11-472-10F

- Hydrolysis units: (See Figure 3)
- Lab jack: Fisher No. 14-673-10
- Stir plate: Fisher No. 14-493-120MR
- Rotary evaporator
- Sargent-Welch high vacuum pump, model 1400B
- Cold finger condenser (45 cm long) filled with dry ice
- Bottle, 1 or 2 oz. for collection of eluent from SPE columns
- Graduated cylinder, 50-100 mL
- Glass wool: Fisher No. 11-390
- Becton-Dickenson disposable syringe with Luer-Lok tip, 3 or 5 ml: Fisher Nos. 14-823-40 or 14-826-12
- Acrodisc HPLC syringe filters, nylon, 0.45 µm: Fisher Cat. No. 09-730-259
- 12 port vacuum manifold for SPE columns with transparent side walls and pressure gauge: Baxter No. 9400-0K
- Florisil SPE columns, 1 g resin, 6 mL reservoir: J. T. Baker No. 7213-07
- Neutral Alumina resin, Brockman Activity I, Fisher No. A950-500
- Amino resin, 40µm, Baker 7028-R
- Decolorizing Carbon
- The Meyer N-EVAP evaporator or equivalent
- 1.8 mL autosampler vials with teflon lined resealable septa and phenolic caps: Varian No. 96-000099-00
- Perkin-Elmer Series 4 gradient LC system
- Perkin-Elmer ISS-100 autosampler
- Brownlee Labs RP-18 guard column, 1.5 cm L x 3.2 mm ID: P. J. Cobert No. 933005
- Stainless steel tubing: 1/16" x 0.010" ID and 1/16" OD x 0.020" ID



Components of the units available from Ace Glass Inc.:

Separatory Funnel (125 mL), Part No. 7229-08
Claissen Adapter, Part No. 5055-10
Allihn Condenser, Part No. 5941-12
Connecting Adapter, Part No. 5125-10
Liebig Condenser, Part No. 5998-12
Trubore Tube, Part No. 8700-46

Figure 3: Hydrolysis Unit

- Waters 470 Scanning Fluorimeter (or equivalent equipped with 280-290 nm excitation filter and 360 and 405 nm emission filter)
- C_{18} Beckman Ultrasphere HPLC column, 10 mm I.d. x 25 cm L, 5 µm particle size: Beckman No. 244046 or Zorbax ODS Ultrasphere HPLC column, 9.4 mm x 25 cm
- Fisher Recordall model 5000 recorder
- Solvent buffer reservoirs

2) REAGENTS

- Acetonitrile, OPTIMA™ Grade: Fisher No. A996-4
- 2,2,4-Trimethylpentane (Isooctane), OPTIMA™ Grade : Fisher No. O301-4
- Ethyl acetate, (EtOAc), OPTIMA™ Grade: Fisher No. E196-4
- Methanol, (MeOH), OPTIMA™ Grade: Fisher No. A454-4
- 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, (dI water).
- Methylene chloride, (CH_2Cl_2), OPTIMA™ Grade: Fisher No. D151-4
- Sodium hydroxide, (NaOH), 50% solution, Certified ACS: Fisher No. SS254-1
- Phosphoric acid, 85%, HPLC Grade: Fisher No. A260-500
- Hydrochloric acid, (HCl), 12 N, (37%), Reagent Grade: Fisher No. A144S-212
- Sodium Chloride

B. SOLUTION PREPARATION

Prepare adequate quantities of all reagents. If it is necessary to change vendor or reagent grade, it may be necessary to recheck the background resulting from the addition of a new reagent. All reagents must be HPLC or pesticide residue grade.

Deionized water from a Milli-Q water purification system or equivalent can be used. 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.

65% (v/v) Acetonitrile/water (Extraction Solvent A)
Using a graduated cylinder, mix thoroughly 350 mL deionized water with 5.0 grams sodium chloride. After the sodium chloride has dissolved, add 650 mL acetonitrile and mix.

20% (v/v) EtOAc / Isooctane
Using a graduated cylinder, mix thoroughly 200 mL EtOAc with 800 mL isooctane

10% (v/v) MeOH / Methylene Chloride
Using a graduated cylinder, mix thoroughly 50 mL MeOH with 450 mL methylene chloride

2.0N NaOH
Using a graduated cylinder, dilute 160 g 50% (w/w) NaOH to 1 liter with deionized water

2.5N NaOH
Using a graduated cylinder, dilute 200 g 50% (w/w) NaOH to 1 liter with deionized water

0.2% Phosphoric acid/water
Using a graduated cylinder, mix thoroughly 2 mL phosphoric acid(85%) with 998 mL deionized water

6 N HCl
Using a graduated cylinder, combine 500 mL 12 N HCl with 500 mL deionized water

C. ANALYTICAL STANDARD SOLUTIONS

Analytical standards are prepared for two purposes: for use in spiking matrices to determine analytical recovery and for calibrating the response of the analyte in the liquid chromatographic detectors. The purity of all standards should be verified prior to preparation of the stock solutions. All standards are stored at <8° C in clean amber glass bottles with screw top lids. Those standards which are used infrequently are referred to as stock solutions. These are considered stable at the storage conditions for up to 5 months. Any standard which is opened on a more frequent basis will be considered a working solution and should be remade from a stock solution approximately every 60 days. Stability of stock solutions (5 months) and working solutions (8 weeks) has been demonstrated in the laboratory.

1.) Fortification Spiking Solutions

Samples will be spiked with MON 37500 at levels as low as 0.001 ppm. Higher fortification levels will depend on the residues found in the soil.

Weigh 0.1000 ± 0.0020 gram of analytical grade MON 37500 into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well to insure complete dissolution. This solution contains 1000 ± 20 µg/mL of MON 37500.

Weigh 0.1000 ± 0.0020 gram of analytical grade sulfonamide into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well to insure complete dissolution. This solution contains 1000 ± 20 µg/mL of sulfonamide.

Pipet 1.0 mL of the 1000 µg/mL MON 37500 solution and 5.0 mL of the 1000 µg/mL sulfonamide solution into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well. This solution contains 10.0 µg/mL of MON 37500 and 50 µg/mL of sulfonamide.

Pipet 10 mL of the 10 µg/mL MON 37500 solution/50µg/mL sulfonamide solution into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well. This solution contains 1.0 µg/mL of MON 37500 and 5.0 µg/mL of sulfonamide.

Pipet 1.0 mL of the 10.0 µg/mL MON 37500 solution/50µg/mL sulfonamide solution into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well. This solution contains 0.10 µg/mL of MON 37500 and 0.50 µg/mL of sulfonamide.

These solutions are adequate to fortify 50.0 gram samples in the range of 0.001 to 1.0 ppm. All fortifications should be made using a volume of the appropriate standard greater than 0.5 mL. Working solutions may be prepared in absolute ethanol instead of acetonitrile, however, due to limited solubility in ethanol, the 1000 µg/mL stock solutions should be prepared in acetonitrile. The following fortification scheme for MON 37500 provides an example:

Volume of 0.1 µg/mL Standard	Amount of MON 37500	Concentration of MON 37500 in 50 g soil sample
0.5 mL	0.05 µg	0.001 ppm
1.0 mL	0.1 µg	0.002 ppm

Volume of 1.0 µg/mL Standard	Amount of MON 37500	Concentration of MON 37500 in 50 g soil sample
0.5 mL	0.5 µg	0.01 ppm
1.0	1.0	0.02
2.0	2.0	0.04

Volume of 10.0 µg/mL Standard	Amount of MON 37500	Concentration of MON 37500 in 50 g soil sample
0.5 mL	5.0 µg	0.10 ppm
1.0	10.0	0.20

2.) Detector Calibration Standards

The detector calibration standards are made at convenient concentrations. Concentration in MON 37500 equivalents is also given. Molecular weight ratios are used to determine equivalencies. The molecular weight of MON 37500 is 470.49 grams/mole. The molecular weight of the rearranged MON 37500 is 363.40 grams/mole and the molecular weight of the sulfonamide is 289.34 grams/mole. One gram of sulfonamide is therefore equivalent to 1.626 grams of MON 37500 and one gram of rearranged MON 37500 is equivalent to 1.295 grams of MON 37500.

Weigh 0.1000± 0.0020 gram of analytical grade sulfonamide into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well to insure complete dissolution. This solution contains 1000 ± 20 µg/mL of sulfonamide.

Weigh 0.1000± 0.0020 gram of analytical grade rerranged MON 37500 into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well to insure complete dissolution. This solution contains 1000 ± 20 µg/mL of rearranged MON 37500.

Pipet 1.0 mL of the 1000 µg/mL standard into a 100 mL volumetric flask, dilute to volume with acetonitrile and mix well. This solution contains 10.0 µg/mL (Separate flasks for each component)

From these working solutions, make the calibration standard solutions. Suggested concentrations are given, however, different concentrations may be substituted as needed. All solutions are diluted to a final volume of 100 mL.

Volume of 10.0 µg/mL Standard	Final Rearr MON 37500 Conc	Final Rearr MON 37500 Conc in MON37500 Eq.	Vol of 10.0 µg/mL Standard	Final Sulfon. Conc.	Final Sulfon. Conc. in MON 37500 Eq.
0.1 mL	0.01 µg/mL	0.01295 µg/mL	0.5 mL	0.05µg/mL	0.0813µg/mL
0.5	0.05	0.06473	2.0	0.20	0.3252
1.0	0.10	0.12947	5.0	0.50	0.8130
2.0	0.20	0.25893	8.0	0.80	1.3008
4.0	0.40	0.51787	12.0	1.20	1.9512
6.0	0.60	0.77680	15.0	1.50	2.4390

D. ANALYTICAL PROCEDURE

1.) Sample Preparation

Matrix samples are taken in the field according to the study protocol. The samples are frozen for shipping, homogenized, and further prepared as appropriate under the specifications of the study protocol.

2.) Sample Analysis

Refer to Figure 2, Method Flowchart, for an overview.

a. Extraction/Filtration

Weigh 50 ± 0.1 grams of soil into a 250 mL centrifuge bottle. Fortifications to the sample must be made at this point by using the correct volume of the appropriate fortification solution. *Allow the fortification solution to warm to room temperature before use.* Add 80 mL of extraction solvent A. Shake for 1 hour on a mechanical shaker. Centrifuge for 20 minutes at 4000 rpm. In order to balance the weight in the centrifuge bottles, add extraction solvent A to the lighter bottle prior to centrifugation. Pour liquid into a 500 mL round bottom flask. Set round bottom flask aside.

Add 40 mL acetonitrile and 40 mL 2N NaOH to the soil in the centrifuge bottle. Shake again for 1 hour and then centrifuge again at 4000 rpm for 20 minutes. Use acetonitrile to balance the weight in the bottles prior to centrifugation. *Note: For soils where extractability is low due to binding, the soil can be left on the shaker overnight to increase extractability. In this case, the soil is centrifuged the next morning. This will be the preferred method for most soils.* Pour the liquid into a 250 mL separatory funnel. Drain the lower aqueous layer into a beaker. Pour the upper layer into the 500 mL round bottom flask which contains the first extract. Pour the aqueous extract back into the separatory funnel.

Add 60 mL acetonitrile to the centrifuge bottle, cap, and shake vigorously by hand for 1 minute. Allow soil to settle. Decant the acetonitrile into the separatory funnel (use a funnel with a glass wool plug to prevent soil from entering the separatory funnel). Shake the separatory funnel gently for 30 seconds and allow the layers to separate. Discard the lower, aqueous layer. Pour the upper layer into the 500 mL flask with the previous extracts.

b. Sample concentration

Reduce the volume in the 500 mL flask to 20-30 mL by rotary evaporation. A water bath can be used to speed up the evaporation of acetonitrile, however, do not heat above 50 °C. This step is needed to remove the acetonitrile. If acetonitrile is left in the reaction flask, it will react with the NaOH to form acetamide. The resulting lower concentration of NaOH will lead to hydrolysis of MON 37500 to sulfonamide.

c. Base hydrolysis

After the sample has been concentrated, add 30 mL 2.5 N NaOH and reflux the sample for 45-60 minutes. *Prior to reflux, a few mL of solution should be distilled in order to assure that all of the acetonitrile has been removed. Then turn on the reflux condenser and proceed with the reflux.* During reflux, some samples will foam into the reflux condenser. Addition of a few mLs of water or increasing the speed of stirring will usually alleviate this problem. Allow the sample to cool, then add 6N HCl to adjust the pH to 5-6. Approximately 12 mL HCl is required.

d. Methylene chloride extraction

When samples have cooled sufficiently, transfer to clean 250 mL separatory funnels. Rinse flasks with 5-10 mL of water and add this to the separatory funnels. Rinse the flasks with methanol to remove excess water. Discard the methanol and allow the flasks to dry. Add 50 mL methylene chloride to the separatory funnels. Cap the funnels, shake gently for approximately 30 seconds, and allow the layers to separate. If the samples are shaken too vigorously, an emulsion will form which can be broken by centrifuging the samples for 5-10 minutes at 4000 rpm. After the layers have separated, drain the lower methylene chloride layer into the 500 mL flask which was used for the base hydrolysis.

Add a second 50 mL portion of methylene chloride and repeat the above extraction. Drain the methylene chloride into the 500 mL flask. Evaporate the samples to dryness by rotary evaporation.

e. Florisil SPE Cleanup

To a factory packed 1 gram florisil column in a 6 mL reservoir add 1 gram of mixed resin (.66 neutral alumina, .33 amino, 0.004 carbon). Add a small plug of glass wool to prevent disturbance of the resin bed. Then condition the column with 5 mL of 10% methanol in methylene chloride, followed by 5-10 mL of isooctane, under a vacuum of 5-10 inches Hg. *During the SPE cleanup procedures described below, do not allow the columns to go dry.*

After the column is conditioned, redissolve the sample in 7-8 mL methylene chloride. Dilute with 2-3 mL isooctane. Pipet the sample onto the florisil column and elute at a rate that individual drops can be counted. Rinse the sample flask with 5 mL isooctane followed by 10 mL 20% ethyl acetate in isooctane. Elute this rinse from the florisil column and combine the eluent with the previous fraction. Discard all fractions collected to this point.

Place a 100 mL round bottom flask or a 2 oz. glass bottle under the florisil column. Elute with 30 mL of 10% methanol in methylene chloride at a rate slow enough to count the drops coming off the column. Evaporate the eluent with either a stream of nitrogen or by rotary evaporation. The sample is now ready for HPLC analysis.

3.) HPLC Analysis

Redissolve sample in 2.0 mL deionized water. The aqueous HPLC mobile phase can be substituted along with a maximum of 10% acetonitrile. Mix thoroughly to insure that the sample has been completely redissolved.

The sample is injected onto a semi-prep C-18 column (25 cm x 10 mm) by means of an autosampler. The sulfonamide is detected with a fluorimeter with excitation wavelength set

at 280 nm and emission wavelength set at 360 nm. The rearranged MON 37500 is detected with excitation wavelength set at 290 nm and emission wavelength set at 405 nm.

A calibration curve is generated for every set of samples. Levels of sulfonamide external standard are prepared in the range of 0.05-1.5 µg/mL. Levels of rearranged MON 37500 external standard are prepared in the range of 0.01-0.60 µg/mL. The standards are placed among the analytical samples in such a way that at least every fourth injection is a standard. The first and last sample in each analytical sample set must be a standard. The calibration curves are generated by plotting the peak height or peak area of the detector response against the concentration of each calibration standard. Least squares estimates of the data points are then used to define the calibration curve. Linear or exponential curve fit may be used at the discretion of the analyst, but consistency must be maintained throughout a protocol.

HPLC Gradient System:

Mobile Phase:

Solvent A: 0.2% phosphoric acid in H_2O
Solvent B: Acetonitrile

Detector: Waters Model 470 fluorimeter (or equivalent);
ex 280 nm, em 360 nm, 0-24min; ex 290nm, em 405 nm, 24-50min

Injection volume: 200 µL

Column: 25 cm x 10 mm Zorbax SB C18

Column and solvent temp: Ambient

Gradient:

		Mobile Phase	
Time	Flow Rate	A	B
0 min	1.0 mL/min	70%	30%
17	1.0	55	45
18	1.0	55	45
38	1.0	0	100
39	1.0	0	100
49	1.0	70	30
50	1.0	70	30

Inject sample with flow rate of 1.0 mL/min, 70% solvent A. After 17 minutes, begin a linear 1 minute gradient to 45% B, then hold at 45% B for 20 minutes. After 38 minutes, begin a linear 1 minute gradient to 100% B, then hold at 100% B for 10 minutes. Forty-nine minutes after injection, begin a 1 minute gradient back to 70% A. Hold at 70% A for 10 minutes to equilibrate before the next injection. Total time between injections is 60 minutes.

E. INTERFERENCES

Using the analytical method described here, very little interference has been observed in the soils tested. However, it is recommended that a soil be screened for interferences prior to analysis since each soil is different. No interferences have been observed when using high purity solvents and reagents.

Before glassware is sent to the dishwasher, it is pre-rinsed with methanol, then water. The glassware cleaning procedure consists of washing in a mechanical washer with hot soapy water followed by deionized water rinse and final acetone rinse. No interferences have been observed.

F. CONFIRMATORY TECHNIQUES

There have been no confirmatory techniques developed at this time for the sulfonamide or the rearranged MON 37500.

G. TIME REQUIRED FOR ANALYSIS

An analytical set is comprised of 6-12 samples: an untreated control, 2 fortified controls, and 3 replicate samples from the treated plot at each sampling depth (ie. 0-6in, 6-12in, etc). A general scheme for sample analysis is sample extraction with acetonitrile/water on day 1; acetonitrile/NaOH extraction overnight; sample concentration, base hydrolysis, and methylene chloride extraction on day 2, sample cleanup and HPLC on day 3. HPLC analysis is then run overnight. A set of 12 samples and 7 standards will require about 19 hours for HPLC analysis.

H. MODIFICATIONS OR POTENTIAL PROBLEMS

Initial sample extraction is tedious and time consuming due to the presence of bound residues, especially at later time points. In initial samples (0 DAT-7DAT) the MON 37500 and metabolites do not bind to the soil and the NaOH extraction may not be needed. However, as time goes on, this step is vital.

Removal of all acetonitrile before base hydrolysis is essential. Three mL of acetonitrile will potentially consume all of the NaOH during reflux. If the pH gets too low during reflux, a competing reaction will occur in which the MON 37500 is rapidly converted to sulfonamide.

Prior to methylene chloride extraction, the sample must be acidified (pH =6). If the sample is basic, the sulfonamide will be left in the aqueous layer. During methylene chloride extraction, an emulsion will sometimes form, especially when samples are shaken vigorously. If this emulsion forms, it can be broken by centrifuging or by filtration.

Some samples are difficult to redissolve after rotary evaporation. The addition of a small amount of organic solvent (methylene chloride prior to florisil cleanup, acetonitrile prior to HPLC analysis) is recommended. For best results, the flask should be swirled in this stronger solvent prior to addition of the weaker solvent.

There are small interferences in the HPLC analysis which can be eliminated by small changes in the excitation and emission wavelengths. If these interferences remain, changes in the mobile phase may be needed. Interferences will vary with soil type. Non-reproducible interferences in the chromatography may occur. Cleanup or replacement of the HPLC column is recommended.

I. PRECISION AND LIMITS OF DETECTION

This analytical method has been validated to a lower limit of 0.001 ppm for MON 37500 and 0.005 ppm for sulfonamide. Standard solutions can be easily quantified at one half this level.

J. CALCULATIONS

The amounts of rearranged MON 37500 and sulfonamide found in the samples represent the amount of MON 37500 and sulfonamide present in the samples. For this reason, all calculations involving the rearranged MON 37500 are carried out as equivalents of MON 37500. When entering standard concentration data for calculations of the calibration curve, parent equivalents are used.

In all calculations, MON 37500 is defined as the parent equivalent of the rearranged MON 37500 being determined.

1.) Linear Calibration

The concentration (in parent equivalents) of the rearranged MON 37500 in the injected sample is determined based upon the height or area of the peak and interpolation of the internal or external standard linear calibration curve as defined according to the following equation:

$(PKR_{analyte})m + b = \mu g/mL$ Analyte

Where,

$(PKR_{analyte})$ is the detector response, as peak height or area of the analyte (ethyl sulfone).

m is the slope of the linear least squares fit of the calibration curve.

b is the Y-intercept of the linear least squares fit of the calibration curve.

NOTE: Due to the limited linear range of the detectors, proper dilution of each sample may be necessary to keep the peak response of the analytes within the limits of the calibration standards. If a sample is found to have a peak response for an analyte greater than the highest calibration standard, it must be diluted with an appropriate solvent. This dilution must be recorded to obtain the total amount of analyte found in the sample for ppm calculation.

The resulting µg/mL value reflects the concentration of MON 37500 equivalents in the injected sample. This value must be multiplied by the dilution volume (usually 2 mL) to obtain the µg MON 37500 extracted from the sample in the first step of the method.

Now that the concentration of rearranged MON 37500, as MON 37500 equivalents, in the injected sample and the dilution volume are known, the total amount of MON 37500 present in the sample can be calculated using the sample weight. Since the HPLC calibration standard curve constants are calculated to provide MON 37500 equivalent values for the µg/mL in the injected samples, no further molecular weight conversions are necessary. The concentration of MON 37500 in the injected sample is multiplied by the dilution volume to obtain the total amount (µg) of MON 37500 in the sample analyzed. This total amount of MON 37500 is divided by the sample weight (grams) of the sample analyzed resulting in µg/gram or parts per million (ppm).

(µg/mL MON 37500) x (dilution volume/sample weight) = ppm MON 37500

2.) Exponential Calibration

Exponential calibration may be used should the analyst desire. The calibration curve is obtained by regression as follows:

$\ln (PKR_{analyte}) = A + B \ln$ (µg/mL Analyte)

Thus,

$\ln$ (µg/mL) = $\{\ln(PKR_{analyte})-A\}/B$

$\ln$ (µg/mL) = $(-A/B) + (1/B)\ln(PKR_{analyte})$

Where,

$(PKR_{analyte})$ is the detector response, as peak height or area, of the analyte.

A is the slope of the curve of the natural log of the concentration and detector response

B is the Y-intercept of the curve of the natural log of the concentration and detector response

Once the µg/mL concentration of the analyte is determined in the injected sample, the remainder of the calculations are the same as the linear calibration curve.

3.) Analytical Recovery

Analytical recovery is determined on a matrix by matrix basis. The analytical recovery for an individual fortification is determined by dividing the ppm of the analyte found minus the average ppm in the control samples by the amount of analyte fortified at the beginning, and

then multiplying by 100%. An example calculation is given here:

0.1 ppm MON 37500 spiked prior to extraction

0.11 ppm parent equiv. of rearranged MON 37500 found in sample after analysis

0.02 ppm parent equiv. of rearranged MON 37500 found in control sample

{(0.11-0.02)/0.1} x 100% = 90% recovery

If the amount of analyte found in the control sample is zero, then the recovery is simply the amount found divided by the amount fortified. Background correction must be completed prior to determining the overall average recovery of a matrix. To determine the overall analytical recovery for a matrix, the individual analytical recoveries of the rearranged MON 37500 are averaged.