1 SCOPE

This analytical method determines residues of the three major carboxylic acid metabolites of MON 15100 in soil.

1.1 INTRODUCTION

Three major metabolites of MON 15100 [3,5-pyridinedicarbothioic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S,S-dimethyl ester] in soil have been identified. Those metabolites are (1) the diacid metabolite [3,5-pyridinedicarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-], (2) the normal monoacid metabolite [3-pyridinecarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-5-[(methylthio)carbonyl]-6-(trifluoromethyl)-] and the reverse mono-acid metabolite [3-pyridinecarboxylic acid, 6-(difluoromethyl)-4-(2-methylpropyl)-5-[(methylthio)carbonyl]-2-(trifluoromethyl)-]. Metabolite structures are shown in Figure 1.

The analytical method described determines the methyl ester derivatives of the three major metabolites of MON 15100 in soil. The method may be summarized by the schematic diagram shown in Figure 2. The soil sample is extracted using an acidic medium in order to suppress ionization of the carboxylic acids. The carboxylic acids are derivatized to methyl esters prior to analysis by gas chromatography with electron capture detection (GC-ECD)

The accuracy of the analytical method is estimated based on recovery of known levels of diacid, normal acid and reverse acid fortified onto untreated soil. The fortified soil is then extracted and analyzed via the outlined method. The method has been validated to a level of 0.010 ppm for each metabolite.

1.2 DETECTABILITY

The Lower Limit of Method Validation (LLMOV) is 0.010 ppm of each metabolite of MON 15100 in soil. The LLMOV is resultant from acceptable recovery of 0.10 µg of each metabolite of MON 15100 fortified onto a 10.0 gram soil sample, i.e., 0.010 ppm for each metabolite.

2 MATERIALS AND REAGENTS

The following materials and reagents are necessary for analysis of MON 15100 metabolites. Substitutions may be made as deemed necessary. However, it is strongly advised that the Aldrich Mini Diazald Apparatus be used for generation of diazomethane. This apparatus may be assembled in a minimum of time and it efficently prevents escape of diazomethane/ether vapor into the surrounding

atmosphere. Diazomethane is extremely toxic and carcinogenic. It also has the potential for violent explosions. Therefore, it should be handled with extreme care and only by those trained in its use. Preparation and use of diazomethane should be conducted in an efficent hood and behind a safety shield.

2.1 MATERIALS AND REAGENTS

Mettler balance, Model PE 3600 or equivalent

Serological pipettes (0.10 mL to 0.50 mL): Fisher No. 13-554 A,B,C

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

10-50 mL Adjustable volume dispenser: American Scientific Products No. P4985-50

Buchner funnel: Fisher No. 10-365B

Filter paper: Fisher No. 09-805B

Vacuum filtration adapter: Aldrich No. Z11,563-0

250 mL Round bottom flask: Fisher No. 10-067-2D

24/40 Ground glass stopper: Fisher No. 14-640J

Calab Rotory Evaporator or equilavent

250 mL separatory funnel: Fisher No. 10-437-11D

Separatory funnel rack: American Scientific Products No. S9192-1

Glas-Col Shaker-In-The-Round equipped with hexagonal head and separatory funnel holders: Fisher No. 14-258, 14-258-1, 14-259

Pastuer pipettes: Fisher No. 13-678-5D

Rubber bulbs: Fisher No. 14-065B

pH Paper: Fisher No. 14-850-12A

Graduated centrifuge tube: Fisher No. 05-538-40A

Aldrich Mini Diazald Apparatus: Aldrich No. Z10,889-8

250 mL Round Bottom flask with Clear-Seal joints: Aldrich No. Z10,036-6

100 mL Round Bottom flask with Clear-Seal joints: Aldrich No. Z10

125 mL Separatory funnel with Clear-Seal joints: Aldrich No. Z10,038-2

Teflon Stopper: Aldrich No. Z10,039-0

Clips: Fisher No. 05-880D

Thermometer: Fisher No. 14-983-17B

Micro pipetter: Rainin No. P-1000

Macro pipetter: Fisher No. 21-318-600

Pipette tips: Rainin No. RT-200

Macropipetter tips: Fisher No. 21-318-602

Vortex mixer: American Scientific Products No. S8223-1

High capacity silica solid phase extraction columns: Fisher No. P47E

SPE Vacuum Manifold: Supelco No. 5-7030

10 mL volumetric flask: Fisher No. 10-209A

25 mL volumetric flask: Fisher No. 10-209B

100 mL volumetric flask: Fisher No. 10-209D

Ground glass stoppers, size 9: Fisher No. 14-641A

Volumetric pipet, Class A, 10 mL: Fisher No. 13-650-2L

Pipet bulb: American Scientific Products No. P5305-3

1.8 mL Autosampler vails with teflon lined septa and phenolic caps: Varian No. 96-000099-00

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

J and W direct flash injector liner for Megabore columns: Catalog No 210-1064

J and W DB-210 fused silica Megabore column, 30 m x 0.53 mm, 1.0 µm film thickness: Catalog No. 125-0232

or

J and W DB-225 fused silica Megabore column, 30 m x 0.54 mm, 1.0 µm film thickness: Catalog No. 125-2232

2.2 REAGENTS

Fisher Optima grade, or solvents of comparable purity, should be used in order to minimize extraneous peaks from solvents during GC-ECD analysis.

Acetonitrile: Fisher Optima Grade, No. A996-4

Hydrochloric Acid: Fisher A144-500

Sodium Hydroxide: Fisher No. SS266-1

Ethyl ether: American Scientific Products No. 0848-500

Sodium sulfate: Fisher No. S421-500

Diazald: Aldrich No. D2,800-0

Absolute Ethanol, reagent grade

Potassium Hydroxide: Fisher No. P-250

Deionized water

2,2,4-Trimethylpentane (Isooctane): Fisher Optima Grade No. 0302-4

Ethyl acetate: Fisher Optima Grade, No. E196-4

Methanol: Fisher Optima Grade, No. A454-4

2.3 REAGENT PREPARATION

2.3.1 95% Acetonitrile/0.2 M HCl

Prepare 0.2 M HCl by adding 16.7 mL of concentrated (12 M) HCl to 980 mL of deionized water. Adjust volume to 1 liter by adding water. Prepare 95% acetonitrile/0.2 M HCl by adding 211 mL of 0.2 M HCl to 4 liters of acetonitrile.

2.3.2 0.02 M NaOH

Prepare 0.02 M NaOH by diluting 20 mL of 1 M NaOH solution to with deionized water.

2.3.3 6 M HCl

Prepare 0.25 liter of 6 M HCl by SLOWLY adding 125 mL of concentrated HCl (12 M) to 125 mL of deionized water.

2.3.4 Diazomethane

Prepare an ethereal solution of diazomethane from Diazald as specified in the Aldrich Technical Information Bulletin Number AL-121. A reproduction of Bulletin Number AL-121 is shown in Figure 1. A solution of 1 gram Diazald to 15 mL of ethyl ether should be used instead of 1 gram Diazald to 9 mL of ethyl ether as stated in the Information Bulletin. Immediately before starting the generation of diazomethane drain any solid diazald that may have fallen to the bottom of the separatory funnel into a small beaker and dispose of properly.

2.3.5 3% Ethylacetate/isooctane

Prepare 3% ethyl acetate/isooctane by adding 3 mL of ethyl acetate to 97 mL of isooctane.

2.3.6 5% Ethylacetate/isooctane

Prepare 5% ethyl acetate/isooctane by adding 5 mL of ethyl acetate to 95 mL of isooctane.

2.3.7 10% Ethylacetate/isooctane

Prepare 10% ethyl acetate/isooctane by adding 20 ml of ethyl acetate to 180 mL of isooctane.

3 PREPARATION OF ANALYTICAL STANDARDS

3.1 FORTIFICATION SOLUTIONS

3.1.1 CONCENTRATIONS OF FORTIFICATION STANDARDS:

100.0 µg/mL
10.0 µg/mL
1.00 µg/mL

Prepare 100 mL of a 100.0 µg/mL standard of 1) diacid 2) normal acid 3) reverse acid in methanol. Weigh 0.0100 g of diacid into a 100 mL class A volumetric flask. Dilute to 100 mL with methanol to yield a solution of diacid containing 100 µg/mL of diacid. In the same manner, prepare 100 mL of a 100 µg/mL solution of normal acid and 100 mL of a 100 µg/mL solution of reverse acid.

Prepare combined standards containing diacid, normal acid and reverse acid from the 100.0 µg/mL standards. The combined standards contain either 10.0 µg/mL or 1.00 µg/mL of each of the metabolites.

Prepare the 10.0 µg/mL combined standard by pipetting 10.0 mL of each of the 100.0 µg/mL standards into a 100 mL class A volumetric flask. Dilute to 100 mL with methanol to yield a solution containing 10.0 µg/mL each of diacid, normal acid and reverse acid.

Prepare the 1.00 µg/mL combined standard by pipetting 1.00 mL of each of the 100 µg/mL standards into a 100 mL class A volumetric flask. Dilute 100 mL with methanol to yield a solution containing 1.00 µg/mL each of diacid, normal acid and reverse acid.

Store standard fortification solutions in properly labeled amber bottles at 0-4°C.

3.1.2 FORTIFICATION PROCEDURE:

Fortify the 10.0 gram soil sample with a combined standard of each of the three metabolites by pipetting the appropriate volume of the combined fortification standard onto the soil sample. The fortification procedure is summarized by the information in the following table.

+ ppm FORTIFIED refers to the fortification amount of each of the metabolites.

* 0.10 mL each of an individual 100.0 µg/mL standard of diacid, normal acid and reverse acid should be pipetted onto the 10.0 gram soil sample in order to fortify the soil with 1.00 ppm of each metabolite.

	ppm + FORTIFIED	µg FORTIFIED	CONCENTRATION of STANDARD (µg/mL)	mL FORTIFIED
	0.01	0.10	1.00	0.10
	0.02	0.20	1.00	0.20
	0.05	0.50	1.00	0.50
	0.10	1.00	10.0	0.10
	0.50	5.00	10.0	0.50
*	1.00	10.0	100.0	0.10

Fortifications greater than 0.010 ppm require dilution in a final volume of 10% ethyl acetate/isooctane greater than 10.0 mL in order to maintain the peak height reponse within the linear response range of the GC calibration standards. Make final volume adjustments according to the information shown in the following table.

ppm FORTIFIED	FINAL VOLUME (mL)
0.010	10.0
0.020	25.0
0.050	25.0
0.10	25.0
0.50	125.0
1.00	250.0

Any treated samples containing metabolite residues that generate a GC response greater than the response of the highest GC calibration standard (i.e., 0.011 µg/mL) must be diluted with 10% ethyl acetate/isooctane. For metabolite residues between 0.10 ppm and 0.50 ppm final volume adjustments of 50.0 mL or 100.0 mL may also be used.

3.2 DETECTOR CALIBRATION STANDARDS

3.2.1 PREPARATION OF METHYL ESTER DERIVATIVES

Pipette 0.10 mL of a 100 µg/mL standard of diacid, normal acid and reverse acid into a 10.0 mL graduated centrifuge tube. Adjust the total volume to 0.5 mL with ethyl ether. Add 0.50 mL of an ethereal solution of diazomethane to the test tube. Mix gently using a vortex mixer. Allow the test tube to stand at room temperature for 30 min as derivatization occurs. Evaporate the solution to dryness under a gentle stream of nitrogen. Pipette 10.0 mL of 10% ethyl acetate/isooctane into the test tube to yield a standard containing 1.0 µg/mL of each of the three methyl ester derivatives.

3.2.2 PREPARATION OF COMBINED METHYL ESTER STANDARDS

Prepare combined standards of the methyl ester derivatives of diacid, normal acid and reverse acid at the concentrations noted in the following table. Prepare each combined standard by pipetting the specified volume of a 1.0 µg/mL combined standard into a 100 mL class A volumetric flask and dilute to 100 mL with 10% ethyl acetate/isooctane. The concentration in µg/mL for each standard solution refers to the µg/mL concentration of each of the metabolites in the respective solutions.

CONCENTRATION of STANDARD (µg/mL)	VOLUME of 1.0 µg/mL STANDARD (mL)
0.001	0.10
0.003	0.30
0.006	0.60
0.009	0.90
0.012	1.20

Store GC calibration standards in properly labeled amber bottles at 0-4°C.

4 SAMPLE PREPARATION AND EXTRACTION

4.1 SAMPLE PREPARATION

Soil samples a: . collected according to the protocol. The samples are stored frozen until analyzed.

4.2 EXTRACTION

Weigh 10.0 to 11.0 grams of soil into a glass bottle. If necessary, make fortification at this step. Add 50 mL of 95% acetonitrile/0.2 M HCl to soil and cap bottle. Shake on a linear reciprocating shaker for 15 minutes. Filter extraction-solvent into a 250 mL round bottom flask using a buchner funnel and a vacuum filtration adapter. Wash bottle and soil filter cake with approximately 50 mL of acetonitrile/ HCl. Collect filtrate into round bottom flask until total volume is approximately 125mL to 150 mL. Concentrate soil extract to 2 to 3 mL using a rotory evaporator and a warm (35°C) water bath. Add 45 mL of 0.02 M NaOH to a 250 mL separatory funnel. Transfer the concentrated soil extract to the separatory funnel using a Pasteur pipette. Rinse round bottom flask with two 2 mL rinses of 0.02 M NaOH and add to separatory funnel. Adjust sample pH to 10 by adding 1 to 1.5 mL of 1 M NaOH to separatory funnel. (Some precipitation from the sample will occur during this time). Add 50 mL of ethyl ether to the separatory funnel and cap with a polyethylene stopper. Shake for 5 minutes on an electrical shaker equipped with separatory funnel holders. After shaking, allow the sample to separate for at least 10 to 15 minutes. Transfer the NaOH layer into a second separatory funnel. Acidify sample to pH 2 by adding 1 to 2 mL of 6M HCl to separatory funnel. Add 50 mL of ethyl ether to separatory funnel and cap with a polyethylene stopper. Shake for 5 minutes. Allow phases to separate. Discard acid layer. Drain ether layer into a 250 mL round bottom flask and follow with approximately 5 mL of ether rinse. Reduce volume to approximately 2 mL using a rotary evaporator and room temperature water bath. Add anhydrous sodium sulfate to dry sample. Transfer sample to conical graduated centrifuge tube. Rinse round bottom flask and sodium sulfate with three 2 mL portions of ether. Add ether rinses to centrifuge tube. Reduce volume to 1 mL under a gentle stream of nitrogen.

4.3 DERIVATIZATION

Add 0.5 mL of an ethereal solution of diazomethane to the soil extract. Cap centrifuge tube with a teflon stopper. Mix gently using a vortex mixer. Let stand at room temperature for 15 minutes as methylation occurs. (Diazomethane derivatizes the free carboxylic

acids to the corresponding methyl esters, as shown by the reaction scheme in Figure 4). Evaporate sample to dryness under a gentle stream of nitrogen. Add 1.0 mL of isooctane and mix using a vortex mixer.

4.4 SOLID PHASE EXTRACTION

Condition a silica high capacity solid phase extraction (SPE) column using: 1) 5 mL of ethyl acetate 2) 10 mL isooctane. Load a 0.25 mL aliquot of the sample onto the SPE column. Rinse the column with: 1) 3 mL of 3% ethyl acetate/isooctane 2) 3 mL 5% ethyl acetate/isooctane. Elute the methyl esters from the column into a 10 mL volumetric flask using 6 mL of 10% ethyl acetate/isooctane. Adjust sample volume to 10.0 mL with 10% ethyl acetate/isooctane.

5 SEPARATION AND DETECTION

A Varian Model 3600 Gas Chromatograph or equivalent with a ^{63}Ni electron capture detector is used for separation and quantitation of the MON 15100 metabolites.

5.1 OPERATING CONDITIONS

A J and W DB-210 column is used for separation of the derivatized MON 15100 metabolites. However, in case of any matrix interferences using the DB-210 column, an alternative column choice would be the DB-225 column. The operating conditions may be adjusted to achieve the desired separation.

* COLUMN: J and W DB-210 fused silica Megabore column, 30 meters by 0.54 mm, 1 µm film thickness.

or

J and W DB-225 fused silica Megabore column, 30 m by 0.54 mm, 1 µm film thickness.

* TEMPERATURE PROGRAM: 100°C for 3 min,
100-210°C at 10 C/min,
210°C for 5 min.

* INJECTOR TEMPERATURE: 225°C

* DETECTOR TEMPERATURE: 300°C

* CARRIER GAS: Nitrogen at 8 mL/min plus 30 mL/min makeup gas.

* INJECTION MODE: J and W Direct-flash glass injector liner.

* INJECTION VOLUME: 4 µL

* DETECTOR ATTENUATION: 32

* DETECTOR RANGE: 10

A standard chromatogram showing separation of a 0.011 µg/mL combined metabolite standard using the specified operating conditions and a DB-210 column is shown in Figure 5.

5.2 DETECTOR CALIBRATION

Five levels of MON 15100 combined metabolite standards are prepared in the concentration range of 0.0010 µg/mL to 0.0120 ug/mL. These standards are placed among the analytical samples during the chromatographic analysis such that the first, the last and every third injection is that of a standard. A linear calibration curve is generated by plotting the peak height of the detector response versus the concentration of each calibration standard. Linear least squares estimates of the data points is used to define the calibration curve. Example calibration curves are shown in Figures 6, 7 and 8.

6 SOIL MOISTURE DETERMINATION

Calculations of the concentrations of MON 15100 metabolite residues are made with respect to the mass of dry soil analyzed. Since the soil is not dried prior to analysis, the percent moisture in each soil sample must be determined.

6.1 PROCEDURE

Weigh a glass container, e.g. a 100 mL beaker, and record weight to a hundredth of a gram. Weigh an aliquot of soil of 10.0 to 11.0 grams and record weight to a hundredth of a gram. Add the weight of the container plus the weight of the soil and record weight. Place the soil and container in a dry heat oven at a temperature of at least 120°C for at least 12 hours. Remove dry soil and container from oven and allow to cool. Weigh the container and the dry soil and record weight. The soil moisture in the sample is equal to the combined weight of the wet soil plus the container minus the weight of the dry soil plus the container (i.e., [(weight of wet soil + weight of container) - (weight of dry soil + weight of container)]. The percent soil moisture is equal to the soil moisture divided by the weight of the wet soil times 100. Calculation of percent soil moisture is illustrated in Section 7.3.

7 CALCULATIONS

Procedures for calculation of the amount of MON 15100 metabolite residues in each sample, the analytical recovery for the sample set, the percent moisture and the parts per million concentration of MON 15100 metabolite residues are discussed in the following sections.

7.1 QUANTITATION OF MON 15100 ACID METABOLITES

The concentrations of MON 15100 metabolites in the soil extract are calculated from the peak height response of the electron capture detector to the metabolite residues in the sample. The concentrations are calculated by interpolation of the external standard linear calibration curve discussed in section 5.2. The linear calibration curve is defined by Equation 1:

$$(PKHT_{metabolite})m + b = \mu g/mL \text{ metabolite} \qquad (1)$$

Where,

PKHT metabolite — is the height of the MON 15100 metabolite peak.

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.

Due to the limited linear response range of the electron capture detector, all samples must be diluted to maintain the peak height response of the electron capture detector within the range of the calibration standards. The LLOMV is obtained in the 10 mL sample volume. However, samples that contain a metabolite concentration that would generate a peak height response higher than the peak height response to the most concentrated standard must be diluted in a volume greater than 10 mL in order to reduce the peak height response to less than that of the most concentrated standard. This dilution is used in the calculation of the total amount of MON 15100 metabolites in the sample in parts per million. Refer to Section 7.4 for an explanation of this calculation.

7.2 ANALYTICAL RECOVERY

The estimated analytical recovery of the set of samples is calculated from the average recovery from the fortified samples according to Equation 2.

$$\frac{\sum|(C_f/A_f) \times 100|}{N_i} \qquad (2)$$

Where C_f is equal to the amount of analyte found in the fortified check sample, A_f is equal to the actual amount of analyte fortified onto the check sample and N_i is equal to the number of fortified samples.

If the check sample is found to contain any MON 15100 metabolite residues, then that amount must be subtracted from the amount recovered from the fortified sample in order to calculate the recovery for that sample. Thus, the variable C_f in Equation 2 should be replaced by the quantity $(C_f - C_b)$, where C_b is the amount of MON 15100 metabolite found in the check sample. This calculation is illustrated in Equation 3.

$$\frac{\sum[(C_f - C_b/A_f) \times 100]}{N_s} \qquad (3)$$

7.3 PERCENT MOISTURE

The amount of moisture contained in a soil sample is determined as described in Section 6.1. The percent moisture for a given sample is calculated as shown in Equation 4.

$$\left[\frac{(\text{Combined wet wt}) - (\text{Combined dry wt})}{(\text{Wet soil wt})}\right] \times 100 = \% \text{ Soil Moisture} \qquad (4)$$

Where,

(Combined Wet Wt) is the weight (g) of the container plus the weight of the soil before drying.

(Combined Dry Wt) is the weight (g) of the container plus the weight of the soil after drying.

(Wet Soil Wt) is the weight (g) of the wet soil before drying.

(% Soil Moisture) is the amount of moisture in the soil expressed as a percentage of the weight of the wet soil.

The percent moisture is used in order to calculate the concentration of MON 15100 metabolite residues based on the dry weight of the soil sample.

7.4 MON 15100 METABOLITE RESIDUES

After determining the concentration of MON 15100 metabolite residues in the sample aliquot, the total amount of each MON 15100 metabolite in the 10 gram sample is calculated. The amount of MON 15100 metabolites is multiplied by the dilution volume (i.e., the total sample volume divided by 0.25) and is divided by the dry weight of the soil sample to generate the µg of MON 15100 metabolite/gram of soil, i.e. parts per million MON 15100 metabolites. This calculation is illustrated in Equation 5.

$$\left(\frac{\mu g\ \text{METABOLITE}}{mL}\right)\left[\frac{(\text{Dil Vol})}{(\text{Dry Soil Wt})}\right] = \text{ppm METABOLITE} \qquad (5)$$

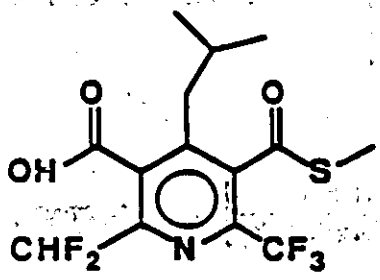
The weight of the dry soil is calculated as shown in Equation 6.

$$(\text{Wet Soil Wt})\left[1 - \left(\frac{\%\ \text{Soil Moisture}}{100}\right)\right] = \text{Dry Soil Wt} \qquad (6)$$

The variables in the equations may be defined as follows.

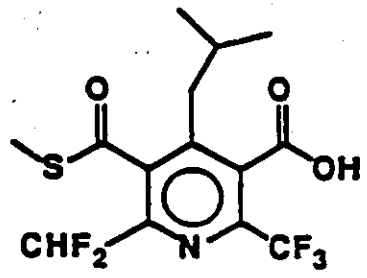
µg metabolite/mL	is the concentration of MON 15100 metabolite in the soil extract, as determined from the linear calibration curve.
(Dil Vol)	is the dilution volume of the extract, taking into account any final dilution necessary to maintain the peak height of the analyte within the range of the highest calibration standard and the 0.25 mL aliquot of the sample analyzed.
(Wet Soil Weight)	is the weight (g) of the soil sample analyzed.
(% Soil Moisture)	is amount of moisture in the soil, expressed as a percentage of the soil weight.
ppm metabolite	is the concentration of MON 15100 metabolite residues in the dry soil, expressed as parts per million.

Figure 1. Chemical Structure of MON 15100 Soil Metabolites



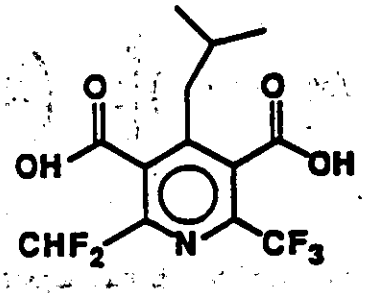
MONO-ACID METABOLITE (NORMAL)

3-pyridinecarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-5-[(methylthio)carbonyl]-6-(trifluoromethyl)-



MONO-ACID METABOLITE (REVERSE)

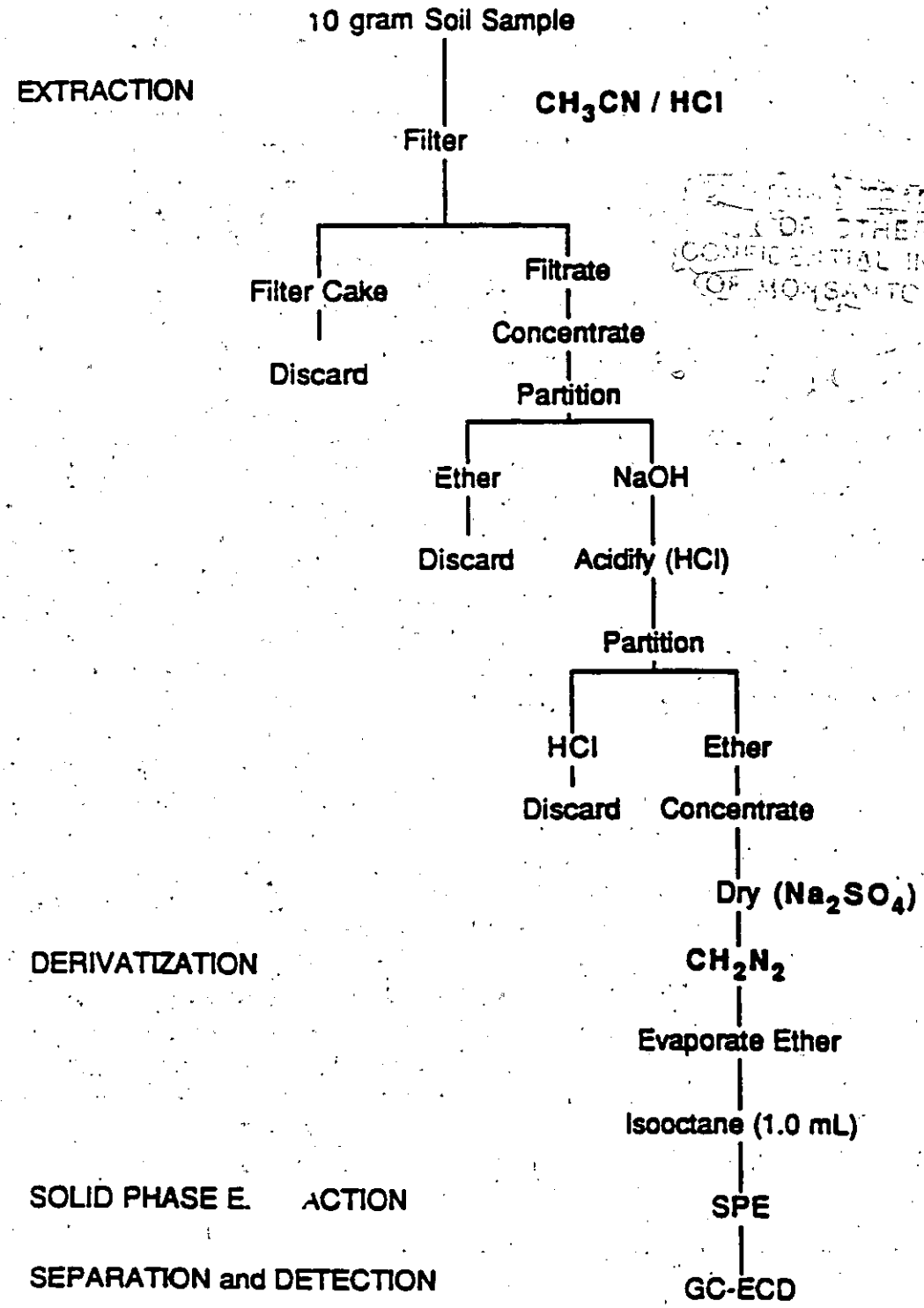
3-pyridinecarboxylic acid, 6-(difluoromethyl)-4-(2-methylpropyl)-5-[(methylthio)carbonyl]-2-(trifluoromethyl)-



DI-ACID METABOLITE

3,5-pyridinedicarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-

Figure 2. Determination of MON 15100 Metabolites In Soil



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PRODUCT NO [illegible]

Technical Information Bulletin
Number AL-121

16

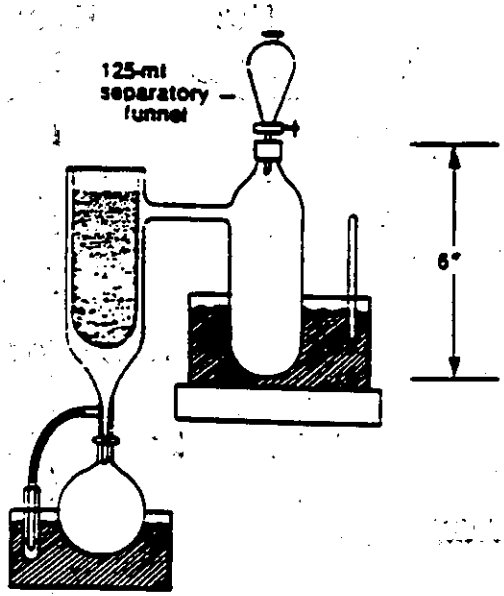
Mini Diazald® Apparatus

The Mini Diazald Apparatus was developed to bridge the gap between the Aldrich MNNG-diazomethane generator (for preparing <1 mmol of diazomethane) and the Diazald Kit (for preparing *ca.* 100 mmol of diazomethane). It consists of a reaction vessel and a condenser in one compact unit. The only other glassware needed are an addition funnel and a receiver flask (must be equipped with Clear-Seal® joints, Note 1). Since both of these pieces are included in our Diazald Kit, the Mini Diazald Apparatus makes a perfect addition to the kit.

The major feature of this apparatus is the cold finger, in place of a water-jacketed condenser. When filled with dry ice/acetone slush, the condenser very efficiently prevents dangerous diazomethane/ether vapor from escaping the apparatus. Nevertheless, it *is* suggested that an ether trap be employed, and that ALL REACTIONS INVOLVING THE PREPARATION AND USE OF DIAZOMETHANE BE CARRIED OUT IN AN EFFICIENT HOOD AND BEHIND A SAFETY SHIELD.

As with all glassware equipped with Clear-Seal joints, this apparatus should be washed very carefully. Wire brushes should not be used since they can scratch the inner surface of the glass.

DIAZOMETHANE PREPARATION



Assemble the equipment as shown. Fill the condenser with dry ice, then add acetone slowly until the cold finger is about one-third full. Add ethanol (95%, 10ml) to a solution of potassium hydroxide (5g) in water (8ml) in the reaction vessel. Attach a 100-ml receiving flask (with Clear-Seal joint) to the condenser and cool receiver in an ice bath. Provide an ice-cooled ether (*ca.* 2ml) trap at the sidearm (the glass rod must have firepolished ends).

Place a separatory funnel (with Clear-Seal joint) over the reaction vessel and charge funnel with a solution of Diazald (5.0g, 23mmol) in ether (45ml). Warm the reaction vessel to 65° with a water bath and add the Diazald solution over a period of 20 minutes. The rate of distillation should approximate the rate of addition. Replenish cold finger with dry ice as necessary. When all the Diazald has been used up, slowly add 30ml of ether and continue the distillation until the distillate is colorless. The ethereal distillate will contain about 700mg (16.6mmol) of diazomethane.

If an alcohol-free ethereal (Note 2) solution of diazomethane is required, add 2-(2-ethoxyethoxy)ethanol (14ml) and ether (8ml) to a solution of potassium hydroxide (2.5g) in water (4ml) in the reaction vessel. Distill diazomethane as in above procedure (a similar yield is obtained).

The Mini Diazald Apparatus works well with as little as 500mg of Diazald. It is not necessary to scale-down the alkali solution. This feature, along with the very efficient condenser, makes the apparatus ideally suited for the preparation of deuterated diazomethane. The reagent quantities outlined in the Deutero-Diazald Prep Set instructions can be used in this Mini Apparatus, up to about 15g of Diazald.

Notes:

1. DO NOT USE A SEPARATORY FUNNEL OR RECEIVER FLASK WITH GROUND-GLASS JOINTS. Glassware without sharp edges or ground-glass joints are recommended to avoid explosions. For the convenience of customers who do not own the Diazald Kit, Aldrich offers the separatory funnel and receivers (three sizes) equipped with Clear-Seal joints.

2. Dioxane and other solvents that may freeze should not be used as the sharp edges of crystals formed may cause an explosion.

Accessories (with ☨19/22 Clear-Seal joints)

Z10,833-1	Round-bottom flask, 50ml, pack of 2
Z10,835-8	Round-bottom flask, 100ml, pack of 2
Z10,836-6	Round-bottom flask, 250ml, pack of 2
Z10,838-2	Separatory funnel with Teflon stopcock, 125ml
Z10,839-0	Teflon stopper, pack of 12
Z10,825-0	Diazald Kit
Z10,851-0	Macro Diazald Set, with ☨24/40 Clear-Seal joints

Please check the current Aldrich Catalog/Handbook for a description of the Diazald Kit and the list of all replacement parts.

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Figure 4. Methylation of MON 15100 Metabolites with Diazomethane