

ANALYTICAL METHOD

SANCOZ CROP PROTECTION CORPORATION

Location: 1300 E. TOWN AVE.
 DES PLAINES, IL 60018

DEVELOPMENT QUALITY CONTROL

Method Number AM-0830-0290-2

Addendum AM-0830-0290-1

Supersedes AM-0830-0889-0

Approved BAB Date 2-26-92

Reviewed _____ Date _____

Reviewed _____ Date _____

Reviewed _____ Date _____

Reviewed _____ Date _____

DETERMINATION OF SAN-582H AND ITS OXALAMIDE METABOLITE IN SOIL

1. SUMMARY

1.1 This method has been developed for the analysis of SAN-582H and its Oxalamide metabolite (see Figure 1. for structures) from soil at concentrations in the sub ppm range. It has been reissued to adequately describe areas where particular attention is required for acceptable recoveries. Recoveries now listed in this report are only from experiments performed using these precautions. No changes have been made in how the method is performed, only in the specifications of steps which must be tightly controlled.

1.2 SAN-582H and the Oxalamide are extracted from soil with 1:1 methanol/aqueous sodium hexametaphosphate, an aliquot is diluted with 1N HCl and partitioned with 1:1 ethyl ether:methylene chloride. The organic phase is concentrated and the Oxalamide is derivatized with diazomethane. SAN-582H and Oxalamide (as the methyl ester) are quantitated by gas chromatography using either a nitrogen phosphorous detector (NPD) and an HP-5 Megabore capillary column or a Mass Selective Detector (MSD) and an HP-1 capillary column. In most cases the MSD was necessary to obtain an adequate signal in relation to background. The Nitrogen phosphorous detector sometimes showed backgrounds that were significantly higher than the stated limit of detection. A comparison of some GC/NPD and GC/MSD results for background and recoveries is given in Table 3.

- 1.3 Sample background, from validation analyses performed with particular attention to all precautions on steps which need tight control, are listed in Table 1. These values are for Gas Chromatographic analysis using the Mass Selective Detector (GC/MSD) since it has been shown that this is the preferred quantitation tool for the best result the first time, without the necessity of confirmation reanalysis. See Table 3 for a comparison of GC/MSD and GC/NPD results.

The highest measured background (signal noise) was 0.002 ppm for Oxalamide and 0.003 ppm for SAN-582H. These values are below the validated limit of detection and are given as indications of expected noise and not to suggest quantitation at this level. Average measured background was 0.0013 ppm (SD=±0.0005, N=4) for Oxalamide with two zero values not included in this average, and 0.0017 ppm (SD=±0.0008, N=6) for SAN-582H with four zero values not included in this average. Five times these noise levels would give a limit of detection of 0.0065 ppm and 0.0085 ppm for Oxalamide and SAN-582H, respectively. The validated limit of detection for both compounds is 0.01 ppm.

2. SAFETY

- 2.1 The oral LD₅₀ of SAN-582H in rats is 1570mg/kg.
- 2.2 Normal laboratory precautions are sufficient for safe handling of SAN-582H.
- 2.3 Ethyl ether is extremely flammable and methanol and toluene are flammable and should not be used near heat, sparks or open flames.
- 2.4 All solvents should be used only in well ventilated laboratories.
- 2.5 Methylene chloride is a suspected carcinogen. Solvent resistant gloves and adequate ventilation should be used when handling methylene chloride.

2.6 Diazomethane and the Diazald[®] reagent are suspected carcinogens. The methylation step should be done in a hood and gloves should be worn.

2.7 Protective glasses should be worn during all laboratory procedures.

2.8 Disposal of samples and standards must be done in compliance with on-site safety policies and procedures.

3. MATERIALS / METHODS

3.1 APPARATUS

3.1.1 Balance, electronic, top-loading, 1000 g capacity, 0.01 g sensitivity.

3.1.2 Balance, electronic, 240 g capacity, 0.1 mg sensitivity.

3.1.3 Bottles, screw cap with Poly-Seal[®] liners, 8-oz.

3.1.4 Centrifuge, 8 oz. capacity (e.g. Damon/IEC Model CU-5000)

3.1.5 Distillation receivers, 15-mL.

3.1.6 Evaporator, nitrogen, (e.g. N-Evap, Organomation Associates, Northborough, MA. 01532)

3.1.7 Funnels, 60°, 75mm.

3.1.8 Gas Chromatograph, Hewlett-Packard model 5890 with Nitrogen Phosphorous Detector (NPD), or similar instrument.

3.1.9 Gas Chromatograph, Hewlett-Packard model 5880 with 5970 Mass Selective Detector (MSD) and autosampler, or similar instrument.

3.1.10 Glass wool, pyrex.

3.1.11 Graduated cylinders, 50-mL.

3.1.12 Kuderna-Danish flasks, 125-mL.

3.1.13 Methylation apparatus (see Figure 2).

3.1.14 Pipets, disposable, 10-mL.

3.1.15 Pipets, volumetric, 1-mL, 2-mL, 5-mL.

- 3.1.16 Separatory funnels, with Teflon[®] stoppers, 125-mL.
- 3.1.17 Vigreux condensers, with 24/40 joint, 38 cm.
- 3.1.18 Volumetric flasks, 100-mL.
- 3.1.19 Water bath, 65 C.

3.2 Reagents

- 3.2.1 Analytical Reference Standards, SAN-582H and Oxalamide, Sandoz Crop Protection.
- 3.2.2 Diazald[®], Aldrich Chemical Co.
- 3.2.3 Carbitol[®], 2-(2-ethoxyethoxy)ethanol, Aldrich Chemical Co.
- 3.2.4 Ethyl ether, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.
- 3.2.5 Hydrochloric acid, concentrated, reagent grade.
- 3.2.6 Methanol, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.
- 3.2.7 Methylene chloride, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.
- 3.2.8 Potassium hydroxide, reagent grade.
- 3.2.9 Sodium Hexametaphosphate, reagent grade.
- 3.2.10 Sodium sulfate, anhydrous granular, reagent grade.
- 3.2.11 Toluene, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.
- 3.2.12 Water, de-ionized, organic-free.

3.3 Preparation of Standards

- 3.3.1 Accurately prepare stock solutions (10⁻⁴g/mL) of 100 mg SAN-582H and Oxalamide in 100 mL methanol in 100-mL volumetric flasks. Accurately prepare a stock solution (10⁻⁴g/mL) of 100 mg Oxalamide Methyl Ester in 100 mL toluene in a 100-mL volumetric flask.

**Dissipation and Mobility of SAN-582H
in Soil After One Fire-emergence
Application to Soybeans in Minnesota
Project 414109, Report #10A Revised**

3.3.2 Transfer 1.0 mL each of 10^4 g/ μ L SAN-582H and Oxalamide stock solutions to a 100-mL volumetric flask and bring to the mark with methanol. This solution (10^4 g/ μ L each in methanol) is used for fortifying check samples.

3.3.3 Transfer 1.0 mL each of 10^4 g/ μ L SAN-582H and Oxalamide Methyl Ester stock solutions to a 100-mL volumetric flask and bring to the mark with toluene (10^4 g/ μ L each in toluene).

3.3.4 Prepare a range of standards for GC/NPD quantitation by dilution of aliquots of 10^4 g/ μ L SAN-582H and Oxalamide Methyl Ester in toluene solutions to 100 mL with toluene in volumetric flasks, as below:

STOCK SOLUTION VOLUME	G.C. SOLUTION CONCENTRATION
20.0mL	2.0×10^2 g/ μ L
10.0mL	1.0×10^3 g/ μ L
5.0mL	5.0×10^4 g/ μ L
2.0mL	2.0×10^5 g/ μ L
1.0mL	1.0×10^6 g/ μ L
0.5mL	5.0×10^6 g/ μ L

3.4 Procedure

3.4.1 Extraction

3.4.1.2 Weigh 50.0 g of soil into an 8-oz. bottle.

If fortifying for recovery determination, add an appropriate volume of fortifying solution (e.g. 500 μ L of 10^4 g/ μ L in 50 g soil gives 0.1 ppm) and evaporate the methanol with a stream of nitrogen.

3.4.1.3 Add 50.0 mL of 50 g/L sodium hexametaphosphate in 0.1N HCL to the sample and shake until soil is dispersed (15 to 45 minutes depending on clay content of soil).

- 3.4.1.4 Add 50.0 mL of methanol to this water/soil mixture and shake for 15 minutes on the reciprocal shaker. Centrifuge at ca. 500 G until the supernatant is clear (15 to 45 minutes depending on soil characteristics).
- 3.4.1.5 With a disposable volumetric pipet, transfer a 10-mL aliquot (5 g equivalent of sample) of the methanolic extract to a 125-mL separatory funnel containing 40 mL of 1M HCl and 50 mL of 1:1 ethyl ether/methylene chloride. The 1:1 ethyl ether/methylene chloride solution must be made up just before it is used and any remainder discarded. If excess solvent mixture is used at a later time the ratio is not the same as originally prepared.
- 3.4.1.6 After equilibration, drain the ethyl ether/methylene chloride (bottom layer) through ca. 10 g of sodium sulfate in a 60° funnel plugged with glass wool, into a Kuderna-Danish flask fitted with 15-mL distillation receiver.
- 3.4.1.7 Add 50 mL of fresh ethyl ether/methylene chloride to the separatory funnel, shake, equilibrate, and drain again through the sodium sulfate.
- 3.4.1.8 Rinse the sodium sulfate three times with 5-10 mL of ethyl ether/methylene chloride, add 1.0 mL of methanol and a boiling chip, fit with a Vigreux condenser, and concentrate to ca. 1-2 mL on the 65°C water bath.

3.4.2 Methylation

- 3.4.2.1 Add 0.5 mL methanol to the concentrated sample and dilute to 5 mL with ethyl ether.
- 3.4.2.2 Fill tube A of the methylation apparatus (see Figure 2) ca. 2/3 full with ethyl ether and establish a nitrogen flow of about 10 mL/min.

3.4.2.3 Add 2 mL of 9N KOH, 2.8 mL of 1:1 Carbitol[®]/ethyl ether, and ca. 100 mg of Diazald[®] to tube B and seat the stopper.

****WARNING****

Diazald[®] is a carcinogen, and the diazomethane gas generated is extremely toxic. Wear gloves and work in an efficiently operating hood. Diazomethane may react violently or explode if the solution contains fine powders or there are ground glass joints or scratched or chipped glassware in the system.

3.4.2.4 Place the distillation receiver containing the sample in position as tube C and pass nitrogen/diazomethane through the sample solution until it turns yellow. If the sample extract has too much color to show the yellow of diazomethane, add ca. 3 mL of ethyl ether to tube D and continue until it turns yellow. It is not necessary to "over Methylate" the samples. When the sample has just turned yellow the sample tube can be removed from the apparatus.

3.4.2.5 Cap the sample tube with a Teflon stopper (do not use ground glass), and allow to stand for 20 min. If the yellow color has disappeared, repeat the addition of diazomethane until the sample just turns yellow. Again, it is not desirable to "over Methylate" the sample. If any yellow color remains additional diazomethane is not necessary.

3.4.2.6 Evaporate the sample extract just to dryness and add 1.0 mL of toluene (or more if high residue levels are present). It is important to make sure the sample is dry, and no residual methanol is present. It is suspected that residual methanol might carry varying amounts of moisture which can participate in hydrolysis of the ester in the hot injector port of the gas Chromatograph. Mix the sample thoroughly and quantitate by GC/MSD, or GC/MSD. GC/MSD has been found to be necessary for many soils because of frequent NPD background interferences.

3.5 Quantitation

3.5.1 Gas Chromatographic Conditions

3.5.1.1 The following instrument and operating conditions have been shown to be suitable for analysis of SAN-582H and Oxalamide in soil. Other conditions may be acceptable provided that SAN-582H and Oxalamide are separated from sample interferences and the response is linear over the range of interest. Elution times and standard linearity must be checked with any new instrument or any change in operating conditions.

3.5.1.2 Instrument: Hewlett-Packard, model 5890 equipped with a 7673A autosampler and a nitrogen phosphorous detector (NPD).

Column: 30 m x 0.53 mm(I.D.) x 0.88 μ m film thickness bonded PSOT HP-5 (5% phenyl methyl silicone).

G.C. Parameters:

Carrier Gas: Helium at 17.6 mL/min. (15 psi head pressure).

Detector Gases:

Hydrogen at 3.2 mL/min.

Air at 92 mL/min.

Helium(Make-Up) at 24.4 mL/min.

Oven Temperature: 180°C

Injector Temperature: 250°C

Detector Temperature: 100°C

Retention Times:

SAN-582H Retention Time: 4.06 min.

Oxalamide Retention Time: 3.65 min.

Retention times will vary slightly with exact G.C. operating conditions and column length and condition.

3.5.1.3 Instrument: Hewlett-Packard 5880A Gas Chromatograph equipped with 5970 Mass Selective Detector and 59970 MS ChemStation software.

Column: 25m x 0.2mm x 0.11um film thickness bonded PSOT HP-1 (methyl silicone).

G.C. Conditions:

Carrier Gas: Helium at 0.4 mL/min. (10 psi head pressure).

Injection Mode: Splitless, 0.5min. purge delay.

Injector Temperature: 250°C

Oven Temperature: 100°C for 0.5 min. then 30°C/min. to 220°C and hold for 2.5 min.

Interface Temperature: 275°C

M.S.D. Parameters:

MSD Mode: Selected Ion Monitoring

Oxalamide Methyl Ester: m/e 240, 213, and 180

SAN-582H: m/e 230, 203, and 154

Retention Times:

Oxalamide Retention Time: 6.47 min.

SAN-582H Retention Time: 6.68 min.

Retention times will vary slightly with exact G.C. operating conditions and column length and condition.

3.5.2 Calculations

3.5.2.1 Prepare standard curves for SAN-582H and Oxalamide by injecting fixed volumes (5 µL for NPD, 2.0 µL for MSD) of a series of standards of known concentrations and plotting peak heights versus concentrations on log-log graph paper.

Page -9-

3.5.2.2 Inject the same volume of sample extract and determine the concentrations of SAN-582H and Oxalamide in the injected aliquot from the peak heights and the standard curves prepared from standards run at the same time.

3.5.2.3 Calculate the concentration of the residues in the sample using the following expression:

$$\text{ppm} = \frac{C \times A \times V}{W}$$

Where:

ppm = Concentration of the analyte in the sample in parts per million ($\mu\text{g/g}$).

C = Concentration of the analyte in the sample extract determined from the standard curve (ng/mL).

V = The volume of the sample extract, taking into account all dilutions (mL).

W = The weight of sample represented by the extract (g).

A = Conversion factor necessary for accommodation of the change in molecular weight of Oxalamide after methylation. ($A=0.94$ for Oxalamide, $A=1$ for SAN-582H)

3.6 Confirmatory Techniques

3.6.1 The use of GC/NPD for quantitation gives frequent false positive results. It should be used in conjunction with the GC/MSD for confirmation, or the GC/MSD can be used exclusively if residues are expected in most samples. In such cases the use of an NPD should be limited to sample screening, and suspected residues should be confirmed by MSD.

3.6.2 The use of MSD in SIM mode monitoring three ions for quantitation includes confirmation. The presence, in the sample, of peaks for all three ions in the same ratios as observed in standards gives extremely high confidence that the sample response is due to the compound of interest.

Page -10-

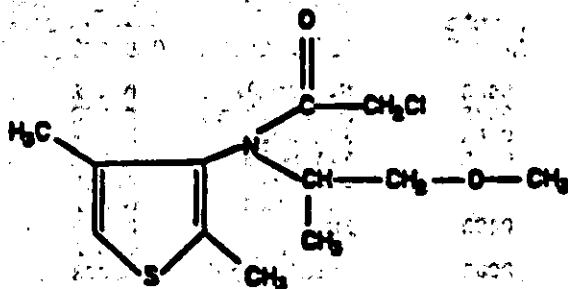
This is reflected in the good agreement between values obtained for each ion when standard curves are prepared for each of the monitored ions and results are calculated as described in section 3.5.2 of this method. When the results for all three ions are similar the average of these three responses may be used to get a single value for the analysis. However, sample co-extractives frequently co-elute and contribute to the response of one or two ions, giving a higher calculated value for those ions. In cases where the result for one ion is much larger than the two remaining ions, and the two remaining results agree closely, the high result may be discounted due to a high contribution from interferences, and the remaining two averaged for a result which still has a high degree of confidence. In cases where there is no close agreement between the results from all three ions, the lowest result may be used on the assumption that co-extractives are contributing to the ions giving the higher results. However, there is no guarantee that co-extractives are not also contributing to the lowest result, and the value obtained has much less confidence.

3.7. Time Required for Analysis

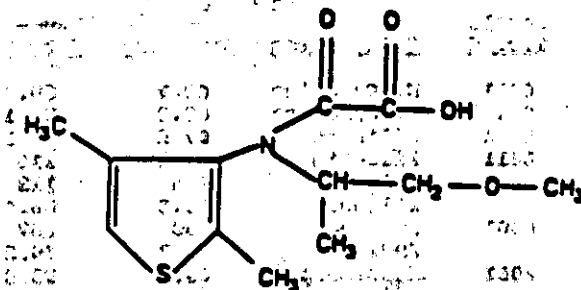
3.7.1

A set of six to eight samples can be prepared in an eight hour day and quantitated overnight using an autosampler. A single sample could be extracted and quantitated in a single eight hour day.

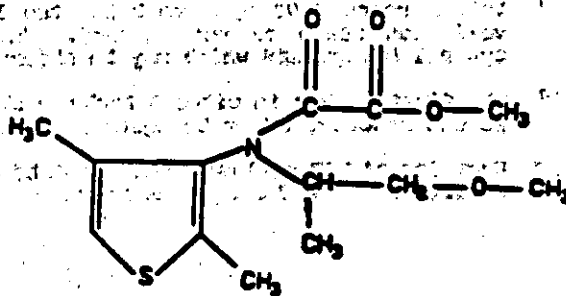
Figure 1. Chemical Structures of SAN-582H and Related Compounds



SAN-582H, 2-chloro-N-(1-methyl-2-methoxyethyl)-N-(2,4-dimethyl-thien-3-yl)-acetamide



Oxalamide, N-(1-methyl-2-methoxyethyl)-N-(2,4-dimethyl-thien-3-yl)-oxalamide



Oxalamide, N-(1-methyl-2-methoxyethyl)-N-(2,4-dimethyl-thien-3-yl)-oxalamide methyl ester