I. SCOPE

The analytical method described here is for the determination of the MON 12000 metabolites, 3-chlorosulfonamide acid (MON 5783, 3-CSAA) and aminopyrimidine (MON 5784), in soil matrices. This method lists the chemicals, solutions, and equipment required for this determination and gives details of the procedures required for the extraction, cleanup, and isolation of these analytes. Typical instrument conditions, example chromatograms, and validation results are also provided.

II. PRINCIPLE

The soil sample is extracted with 75% acetonitrile/25% water followed by evaporation to remove the acetonitrile. Following the evaporation, the remaining aqueous solution is adjusted to a pH >10 and extracted with methylene chloride to isolate the aminopyrimidine. After this extraction the aqueous phase is adjusted to approximately pH 2 and the 3-chlorosulfonamide acid extracted into ethyl acetate. The aminopyrimidine fraction is cleaned up using an amino SPE column and analyzed by GC/TSD. The 3-chlorosulfonamide acid is derivatized with TMS-diazomethane, cleaned up using a silica SPE column, and analyzed by GC/ECD.

III. EQUIPMENT

The following equipment is used in this analytical method. Specific brands are listed, but in most cases equivalent equipment obtained from other vendors can be used.

Gas Chromatograph - Varian 3700 equipped with TSD and ECD detectors and Model 8000 Autosamplers.

Glass Capillary Column - SPB-1, 0.75 mm ID, 1 µm film, 60 meters, Supelco catalog number 2-3720M.

Glass Capillary Column - SPB-35, 0.75 mm ID, 1 µm film, 60 meters, Supelco catalog number 2-3730 (An example of a suitable equivalent column is a J&W 30 meter DB-17 column, 0.53 mm ID, 1 mm film, catalog number 125-1732).

Strip Chart Recorder - Recordall Series 5000, Fisher Scientific catalog number 13-939-20.

Data Aquisition System - A system having appropriate integration and graphics capability. The Monsanto Automated Chromatography System (MACS) was used.

Autosampler Vials - Varian catalog number 03-949835-01.

Analytical Balance - Sartorius AC210S.

Laboratory Balance - Sartorius LC2200S.

DuPont/Sorvall RC-5B Refrigerated Centrifuge, catalog number 50253.

Rotary Evaporator System - Based upon Rinco Rotating Shaft Evaporator, Fisher Scientific catalog number 09-548-100; Kontes Vacuum Trap, Fisher K9269101000; Hot plate, Fisher HP-18325; Water Baths, Cole-Parmer L-07274-00; and Vacuum Pump, Fisher 01-096.

Two-Speed Shaker - Eberbach, Baxter Scientific catalog number S1105.

Utility Carrier for Shaker - Eberbach, Baxter Scientific catalog number S1110.

Laboratory Drying Oven - IsoTemp Model 506G, Fisher Scientific catalog number 13-245-506G.

Bottle-Top Dispensers - Brinkmann Dispensette, Baxter Scientific catalog numbers: 10 mL, P4985-10; 50 mL, P4985-50; and 100 mL, P4985-100.

Glas-Col Benchtop Shaker - Baxter Scientific catalog number S1063-1.

Hexagonal Head, Glas-Col Shaker - Baxter Scientific catalog number S1063-10.

Separatory Funnel Holders, Glas-Col Shaker (2) - Baxter Scientific catalog number S1063-21.

Polypropylene Centrifuge Bottles - Nalgene, Fisher Scientific catalog number 05-562-23.

Sterile Sampler Spatulas - Fisher Scientific catalog number 14-358-20.

Glass Powder Funnels - 80 mm Kimax, Baxter Scientific catalog number F7417-2.

Separatory Funnels - 250 mL Kimax, Baxter Scientific catalog number F7860-250.

Round Bottom Flasks - 500 mL Kimax, Fisher Scientific catalog number 10-067-2F.

Round Bottom Flasks - 250 mL Kimax, Fisher Scientific catalog number 10-067-2D.

pH Indicator Strips - 0-6 Range, Baxter Scientific catalog number P1119-5A.

pH Indicator Strips - 7-14 Range, Baxter Scientific catalog number P1119-7A.

Splash-Guard Adapter - Aldrich, 24/40, 250 mL, Aldrich catalog number Z14779-6.

SPE Vacuum Manifold - Supelco catalog number 5-7030M.

Teflon Solvent Guide Needles - Supelco catalog number 5-7047M.

SPE Columns - Analytichem Amino, 5 grams, P. J. Cobert catalog number 953162.

SPE Columns - Econo-Pac, empty, Bio-Rad catalog number 732-1010.

Magnetic Stirrers - Baxter Scientific catalog number S8275-1.

Magnetic Stirring Bars - Baxter Scientific catalog number S8311-72.

Graduated Cylinders - 10-1000 mL, Fisher Scientific catalog numbers 08-549-5B,C,D,E,G,H,I

Erlenmeyer Flasks - 250 mL, Fisher Scientific catalog number 10-039F.

Disposable Pasteur Pipettes - 5.75 inch, Fisher Scientific catalog number 13-678-6A.

Disposable Pasteur Pipettes - 9 inch, Fisher Scientific catalog number 13-678-6B.

Volumetric Flasks - 100 mL, Fisher Scientific catalog number 10-210C.

Serological Disposable Pipettes - 0.1-10.0 mL, Fisher Scientific catalog numbers 13-678-[illegible]A,B,C,D,E,F,G.

Glass Wool - Pyrex, Fisher Scientific catalog number 11-388.

Teflon Wash Bottles - 500 mL, Baxter Scientific catalog number B7690-500.

Clamps, spatulas, tubing, and other supplies as required.

IV. REAGENTS AND SOLUTIONS

The following reagents are used in this analytical method. Specific brands are listed, but in most cases equivalent reagents from other vendors can be used. It is important to use high quality reagents to avoid chromatographic interferences.

Acetonitrile - Burdick & Jackson, High Purity, Baxter Scientific catalog number 015-4 DK.

Methylene Chloride - Burdick & Jackson, High Purity, Baxter Scientific catalog number 300-4 DK.

Methanol - Burdick & Jackson, High Purity, Baxter Scientific catalog number 230-4 DK.

Isooctane - Burdick & Jackson, High Purity, Baxter Scientific catalog number 362-4 DK.

Ethyl Acetate - Burdick & Jackson, High Purity, Baxter Scientific catalog number 100-4 DK.

Acetone - Burdick & Jackson, High Purity, Baxter Scientific catalog number 010-4 DK.

Deionized Water - Burdick & Jackson, High Purity, Baxter Scientific catalog number 365-4 DK, or deionized water from Millipore Compact MQ+ system, catalog number ZD5211584.

Trimethylsilyldiazomethane - 2 M solution in hexane, Aldrich catalog number 36-283-2; or American Tokyo Kasei, Inc. (TCI), catalog number T1146 (TCI product is 1 M).

Celite Filter Aid - Fisher Scientific catalog number C211-500.

Sea Sand - Fisher Scientific catalog number S25-3.

Analytical Filter Paper Pulp - Fisher Scientific catalog number 09-947.

Silica Gel - For column chromatography, 230-400 mesh, Baxter Scientific catalog number C4582-85

Anhydrous Sodium Sulfate - Certified, Fisher Scientific catalog number S421-500.

Sulfuric Acid, Concentrated - Reagent ACS, Fisher Scientific catalog number A300-500.

2 N Sulfuric Acid - Prepared by dilution of concentrated sulfuric acid (36 N) with high purity deionized water.

Hydrochloric Acid, Concentrated - Reagent ACS, Fisher Scientific catalog number A144-500

0.1 N Hydrochloric Acid - Prepared by dilution of concentrated hydrochloric acid (12 N) with high purity deionized water.

2.5 N Sodium Hydroxide - Certified, Fisher Scientific catalog number SS414-1.

75% Acetonitrile/25% water (V/V).

80% Isooctane/20% Ethyl Acetate (V/V).

70% Isooctane/30% Ethyl Acetate (V/V).

3-Chlorosulfonamide Acid (3-CSAA)- 1 H Pyrazole-4-carboxylic acid, 5-(aminosulfonyl)-3-chloro-1-methyl-, of known analytical purity (If purity is less than 95%, purity correction should be made).

N,N-Dimethylaminochlorosulfonamide AcidMethyl Ester - 1 H Pyrazole-4-carboxylic acid, 3-chloro-5-[(di-methylamino)sulfonyl]-1-methyl-, methyl ester, of known analytical purity (If purity is less than 95%, purity correction should be made).

Aminopyrimidine - Pyrimidinamine, 4,6-dimethoxy-, of known analytical purity (If purity is less than 95%, purity correction should be made).

V. PREPARATION OF ANALYTICAL STANDARDS

1. Fortification Spiking Solutions

3-Chlorosulfonamide Acid

Stock Fortification Standard - This standard is prepared by weighing 0.0100 ± 0.0003 g of 3-Chlorosulfonamide Acid into a 100 mL volumetric flask. The standard is diluted to the volumetric mark with acetonitrile. This stock solution contains 100 µg/mL of 3-Chlorosulfonamide Acid.

3-Chlorosulfonamide Acid Working Standards

1. Dilute 7.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 7.5 µg/mL solution of 3-CSAA.

2. Dilute 6.0 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 6.0 µg/mL solution of 3-CSAA.

3. Dilute 4.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 4.5 µg/mL solution of 3-CSAA.

4. Dilute 3.0 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 3.0 µg/mL solution of 3-CSAA.

5. Dilute 1.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 1.5 µg/mL solution of 3-CSAA.

6. Dilute 10 mL of the 1.5 µg/mL solution from 5 above to 100 mL with acetonitrile in a volumetric flask to provide a 0.15 µg/mL solution of 3-CSAA.

Aminopyrimidine

Stock Fortification Standard - This standard is prepared by weighing 0.0100 ± 0.0003 g of Aminopyrimidine into a 100 mL volumetric flask. The standard is diluted to the volumetric mark with acetonitrile. This stock solution contains 100 µg/mL of Aminopyrimidine

Aminopyrimidine Working Standards

1. Dilute 7.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 7.5 µg/mL solution of Aminopyrimidine.

2. Dilute 6.0 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 6.0 µg/mL solution of Aminopyrimidine.

3. Dilute 4.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 4.5 µg/mL solution of Aminopyrimidine.

4. Dilute 3.0 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 3.0 µg/mL solution of Aminopyrimidine.

5. Dilute 1.5 mL of the 100 µg/mL stock solution to 100 mL with acetonitrile in a volumetric flask to provide a 1.5 µg/mL solution of Aminopyrimidine.

6. Dilute 10 mL of the 1.5 µg/mL solution from 5 above to 100 mL with acetonitrile in a volumetric flask to provide a 0.15 µg/mL solution of Aminopyrimidine.

Store prepared standards, stock and working, in amber bottles at 0 - 6° C.

2. Detector Calibration Standards

N,N-Dimethylaminochlorosulfonamide Acid, Methyl Ester

Stock Calibration Standard - This standard is prepared by weighing 0.01190 ± 0.0003 g of N,N-Dimethylaminochlorosulfonamide Acid, Methyl Ester (MON 5785, ME3CSAA) into a 100 mL volumetric flask. The standard is diluted to the volumetric mark with 20% ethyl acetate in isooctane. This stock solution contains 100 µg/mL of ME3CSAA expressed as 3-CSAA equivalents. Ten mL of this solution is diluted to 100 mL with 20% ethyl acetate in isooctane in a second volumetric flask to provide a stock solution containing 10 µg/mL in 3-CSAA equivalents. One mL of the 100 µg/mL stock solution is transferred to a third volumetric flask and diluted to 100 mL with 20% ethyl acetate in isooctane to provide a 1 µg/mL solution in 3-CSAA equivalents.

ME3CSAA Working Standards

1. Dilute 0.5 mL of the 1 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.005 µg/mL equivalent solution of 3-CSAA

2. Dilute 1 mL of the 1 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.010 µg/mL equivalent solution of 3-CSAA

3. Dilute 1.5 mL of the 1 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.015 µg/mL equivalent solution of 3-CSAA

4. Dilute 2.0 mL of the 1 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.020 µg/mL equivalent solution of 3-CSAA

5. Dilute 0.5 mL of the 10 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.050 µg/mL equivalent solution of 3-CSAA

6. Dilute 0.75 mL of the 10 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.075 µg/mL equivalent solution of 3-CSAA.

7. Dilute 1.0 mL of the 10 µg/mL stock solution to 100 mL with 80% isooctane/20% ethyl acetate in a volumetric flask to provide a 0.10 µg/mL equivalent solution of 3-CSAA.

Aminopyrimidine

Stock Calibration Standard - This standard is prepared by weighing 0.0200 ± 0.0003 g of Aminopyrimidine into a 100 mL volumetric flask. The standard is diluted to the volumetric mark with isooctane. Mix well by repeated inversions and shaking. This stock solution contains 200 µg/mL of the Aminopyrimidine. Ten mL of this solution is diluted to 100 mL with isooctane in a second volumetric flask to provide a stock solution containing 20 µg/mL of Aminopyrimidine.

Aminopyrimidine Working Standards

1. Dilute 5 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 1.0 µg/mL solution of Aminopyrimidine.

2. Dilute 4 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.8 µg/mL solution of Aminopyrimidine.

3. Dilute 2.5 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.5 µg/mL solution of Aminopyrimidine.

4. Dilute 1.0 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.2 µg/mL solution of Aminopyrimidine.

5. Dilute 0.75 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.15 µg/mL solution of Aminopyrimidine.

6. Dilute 0.5 mL of the 20 µg/mL stock solution to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.10 µg/mL solution of Aminopyrimidine.

7. Dilute 10 mL of the 0.5 µg/mL solution from 3 above to 100 mL with 98% isooctane/2% ethyl acetate in a volumetric flask to provide a 0.05 µg/mL solution of Aminopyrimidine.

Store prepared standards, stock and working, in amber bottles at 0-5° C (See Section VI)

VI ANALYTICAL PROCEDURE

1. Sample Preparation

Soil samples are received and stored frozen until just prior to analysis. The soil samples have previously been properly separated, composited, and mixed by the sample preparation facility. The soil cores also were recored where necessary.

2. Sample Extraction

Weigh 75 ± 0.1 grams of the previously prepared soil sample into a 250 mL Nalgene centrifuge bottle. If fortification is required, pipette the appropriate fortifications of aminopyrimidine and 3-chlorosulfonamide acid (3-CSAA) directly onto the soil at this point (See Section XI). Add 130 mL of 75% acetonitrile/25% water solution. Then add 15 mL of celite and 25 mL of sea sand to each sample. Cap tightly and shake vigorously. All samples are prepared in a like manner. Place the centrifuge bottles in the shaker horizontally and shake at the high setting for 15 minutes. Following this, place the centrifuge bottles in the centrifuge and centrifuge for 10 minutes at 10,000 RPM. A small portion of glass wool is placed in an 80 mm powder funnel and each extract is filtered through a similarly prepared funnel into a 500 mL round bottom flask. The glass wool is rinsed with a small portion of 75% acetonitrile/25% water from a Teflon wash bottle.

For certain soils when the samples cannot be satisfactorily decanted after the centrifugation, it is necessary to add approximately 1-1.3 grams of ash-free analytical filter paper pulp (Fisher catalog number 09-947) to facilitate centrifugation and decantation. When this is necessary, the filter paper pulp is added following the addition of the celite and sea sand. The samples also are centrifuges at 11,000 RPM when the addition of filter paper pulp is necessary.

For the second extraction, add 100 mL of 75% acetonitrile/25% water to the soil sample and shake vigorously by hand to break up the packed soil from the first extraction. Again place the samples on the shaker and shake at the high setting for 15 minutes. Following this, the samples are again centrifuged for 10 minutes at 10,000 RPM and the extracts are decanted through the same powder funnels plugged with glass wool and into the respective round bottom flasks. The glass wool and funnel sides are rinsed sparingly three times with 75% acetonitrile/25% water using a wash bottle and the samples treated as described in the following section.

3. Evaporation and Separation of Aminopyrimidine

The combined extracts from Part 2 are evaporated on the rotary evaporators with minimal applied heat (to maintain ambient temperature) to approximately 40-50 mL (to remove acetonitrile). Condensation will generally be observed in the splash guard traps at this point. The remaining aqueous solution is transferred to a 250 mL separatory funnel and the round bottom flasks rinsed twice sparingly with deionized water, which is also added to the solution in the separatory funnel. Two drops or more of 2.5 N sodium hydroxide solution are added and the pH is checked with indicator strips to assure that it is >10 (Additional sodium hydroxide may be required to bring some soil samples to pH). This basic aqueous solution is then extracted by shaking for 4 minutes with a 50 mL portion of methylene chloride. If the phases do not separate well, it may be necessary to add 5-10 mL of saturated sodium chloride solution to facilitate separation of the phases. After the phases have separated, the methylene chloride layer is filtered through an 80 mm powder funnel plugged with glass wool and containing 15 mL of anhydrous sodium sulfate. The dryed organic layer is collected in a 250 mL round bottom flask. The aqueous layer is then extracted in an identical manner with a second 50 mL portion of methylene chloride, which is filtered through the same sodium sulfate into the same 250 mL round bottom flask. The sodium sulfate is rinsed three times with sparing amounts of methylene chloride and the rinses collected in the same 250 mL round bottom flask. The combined dried methylene chloride solution contains the aminopyrimidine, if present, and is treated as described below in Part 5. The remaining aqueous solution is treated as described in Part 4 immediately below.

4. Isolation of 3-Chlorosulfonamide Acid

To the aqueous solution from Part 3 is added approximately 0.7-1.0 mL (Use pH as a guide) of 2 N sulfuric acid and the pH checked with indicator strips to assure that it is 2 or below. This acidic aqueous solution is then extracted by shaking for 4 minutes with a 50 mL portion of ethyl acetate. After the phases have separated, the lower aqueous phase is drawn off into a 250 mL Erlenmeyer flask and saved for the second extraction. The ethyl acetate phase is filtered through an 80 mm powder funnel plugged with glass wool and containing 15 mL of anhydrous sodium sulfate. The dryed organic layer is collected in a 250 mL round bottom flask. The aqueous layer is returned to the separatory funnel, the Erlenmeyer flask rinsed twice with sparing amounts of deionized water and the rinses added to the aqueous phase in the separatory funnel. The aqueous layer is then extracted in an identical manner with a second 50 mL portion of ethyl acetate. The lower aqueous layer is drawn off and discarded, and the ethyl acetate layer filtered through the same sodium sulfate into the same 250 mL round bottom flask. The sodium sulfate is rinsed three times with sparing amounts of ethyl acetate and the rinses collected in the same 250 mL round bottom flask. The combined, dried ethyl acetate solution contains the 3-chlorosulfonamide acid, if present, and is treated as described below in Part 6.

5. Cleanup of Aminopyrimidine Samples

To the methylene chloride solution from Part 3 is added 50 mL of isooctane and the solution evaporated at ice bath temperature on the rotary evaporator down to a final volume of about 40 mL or less. The aminopyrimidine is somewhat volatile and care must be taken to assure during the handling of these samples that analyte is not lost through volatilization. Five gram amino SPE columns are preconditioned with solvents by eluting with 20 mL of 30% ethyl acetate/70% isooctane followed by 40 mL of isooctane. Following this the sample solution from above is added to the column and the liquid allowed to elute just to the level of the column. Solvents eluting to this point are discarded. A 250 mL round bottom flask is placed under the SPE column and the column eluted with 150 mL of 20% ethyl acetate/80% isooctane. The flow rate of the columns is adjusted to a reasonable rate where individual drops of solvent are still visible and the level of liquid is not allowed to drop below the level of the solid column packing (columns should not be allowed to run dry). This fraction is then evaporated with ice on the rotary evaporator to just less than 5 mL (approximately 2-4 mL). The solution is then transferred to a graduated centrifuge tube and the volume adjusted to an appropriate final volume with isooctane rinses of the 250 mL round bottom flask. Dilutions are made as appropriate to keep the analyte concentration within the detector calibration range. For example, a 0.02 ppm fortification sample would have a final volume of 5 mL, while a sample containing 0.10 ppm would have a final volume of 20 mL. This fraction contains the aminopyrimidine and is analyzed as described below.

6. Derivatization and Cleanup of 3-Chlorosulfonamide Acid

The ethyl acetate solutions from Part 4 are evaporated just to dryness on the rotary evaporators. The sample is derivatized by first rinsing down the sides of the 250 mL round bottom flask with 5 mL of acetone. Then, in order, the following are added to each sample: 5 mL methanol, 0.2 mL of 0.1 N hydrochloric acid, and 1 mL of TMS-diazomethane. The samples are mixed well by swirling, stoppered loosely (not tightened), and allowed to stand overnight at ambient temperature. If the samples have lost the deep yellow color by morning, an additional 1 mL of TMS-diazomethane is added and the samples allowed to stand an additional hour. This is sometimes difficult to tell because the sample itself is somewhat yellow, but the colors can be distinguished with experience.

The derivatized sample is then evaporated to dryness on the rotary evaporator and the residue dissolved in 2 mL of ethyl acetate. Ten mL of isooctane are added and mixed well with brisk swirling just before the silica column cleanup. The silica columns are prepared by weighing 5 ± 0.1 gm of silica into blank SPE columns and preconditioning with 20 mL of 30% ethyl acetate/70% isooctane followed by 20 mL of 20% ethyl acetate/80% isooctane. The eluting solvents are allowed to elute just to the level of the silica between solvent fractions. The sample from above (with the isooctane added and well mixed) is added to the column and the solution level allowed to just reach the level of the silica. The column flow rates are adjusted to a reasonable rate at which drops are still visible and the solvent is allowed to just enter the column between solvent fractions (columns should not be allowed to run dry during this cleanup process). All solvents eluting to this point are discarded. A 250 mL round bottom flask is placed under the silica SPE column and the column eluted with 100 mL of 30% ethyl acetate/70% isooctane. The sample is evaporated just to dryness on the rotary evaporator and redissolved in an appropriate dilution of 20% ethyl acetate/80% isooctane. For example, a 0.02 ppm fortification would have a final sample volume of 25 mL, while a sample containing 0.10 ppm would have a final sample volume of 200 mL. This fraction contains the trimethyl derivative of 3-chlorosulfonamide acid and is analyzed as described below.

7. Sample Analysis

The samples are analyzed by gas chromatography using a nitrogen specific detector (TSD or NPD) for the aminopyrimidine and an ECD detector for the trimethyl derivative of 3-chlorosulfonamide acid. Injections are made using an autosampler. Instrument operating parameters are provided below in section VII.

8. Soil Moisture Determination

When the soil samples are also being analyzed for MON 12000 parent and a soil moisture determination is done concurrently with that analysis, it is not necessary to repeat the determination when analyzing for the metabolites.

Calibrate and zero the balance. Weigh a glass container, such as a 100 mL beaker, and record this weight to a hundredth of a gram. Next, weigh an aliquot of 10.00 to 11.00 g of the soil sample to be analyzed into this container, and record this combined weight to a hundredth of a gram. Place the container plus soil in a dry heat oven set to at least 110° C overnight. After drying, remove the container and allow it to cool. Reweigh the container plus the dry soil and record the weight to a hundredth of a gram. Calculate the % moisture as the loss in weight of the sample divided by the original undried sample weight (X100).

VII. INSTRUMENTATION

The analytes, aminopyrimidine and 3-chlorosulfonamide acid (trimethyl derivative) are quantified by capillary gas chromatography using a nitrogen specific detector (NPD or TSD) and an electron capture detector (ECD). Details of the operating parameters are as follows:

Electron Capture Detector (ECD)

(3-Chlorosulfonamide Acid Derivative - analysis as N,N-dimethyl-3-chlorosulfonamide methyl ester)

Varian 3700 Gas Chromatograph/Model 8000 Autosampler.
Supelco SPB-35 Glass Capillary Column, 0.75 mm ID, 1 µm film, 60 meters, or J&W DB-[illegible] mm ID, 1 µm film, 30 meters.

Program: 170° C (1 min); 5° C/min to 270° C (4 min hold).
Injector Temperature: 250°C
Detector Temperature: 300° C
8 psi UHP Nitrogen
3 µL Injection
10 x 32 Attenuation

Thermionic Specific Detector (TSD or NPD)
(Aminopyrimidine analysis)

Varian 3700 Gas Chromatograph/Model 8000 Autosampler.
Supelco SPB-1 Glass Capillary Column, 0.75 mm ID, 1 µm film, 60 meters.
Program: 150° C (4 min); 10° C/min to 280° C (5 min hold).
Injector Temperature: 250°C
Detector Temperature: 300° C
8 psi UHP Nitrogen
3 µL Injection
1 x 32 Attenuation

These conditions are approximate and may vary from instrument to instrument. Similar, but different equipment or column conditions may be used, but may require modification of these parameters.

VIII. INTERFERENCES

Detailed interference studies have not been performed. However, no interference due to solvents or labware has been observed. Example chromatograms are presented in Appendix 1.

IX. CONFIRMATORY TECHNIQUES

No confirmatory method is currently available.

X. TIME REQUIRED FOR ANALYSIS

A set of 12 samples requires approximately 2.5 days from initial extraction to setting the samples up for gas chromatographic analysis.

XI. MODIFICATIONS OR POTENTIAL PROBLEMS

Control and fortified samples should be run in the same analytical set as treated samples. Fortification solutions should be stored at 0-6 ° C with only small volumes removed as needed for immediate requirements. Fresh working standards should be prepared monthly. Stock solutions have been demonstrated to be stable for at least three months, when stored frozen and unopened. Since the solutions must be allowed to warm to ambient temperature before use, an appropriate aliquot may be pipetted into a small glass vial. The aliquot may then be accurately pipetted after warming to ambient temperature. The unused portion of the warmed solution is discarded.

XII. METHOD OF CALCULATION

The amount of each analyte is determined based upon external standard calibration. A non-weighted linear least squares estimate of the calibration curve is used to calculate the amount of analyte in the unknowns. 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. The results are reported in ppm of 3-CSAA for the 3-chlorosulfonamide acid and in ppm of Aminopyrimidine.

When the detector calibration standards are prepared as described in Section V, part 2 for N,N-Dimethylaminochlorosulfonamide acid, methyl ester, the analytical results calculated directly will be in ppm of 3-CSAA. If the standards are prepared based upon the dimethyl methyl ester derivative, then a correction factor will need to be applied. The molecular weight of the trimethyl derivative of 3-chlorosulfonamide acid is 281.7 and that of the 3-chlorosulfonamide acid is 239.6. The factor for the conversion of the trimethyl derivative to the desired analyte, 3-CSAA, would then be 239.6/281.7 or 0.851.

XIII. VALIDATION LIMITS OF METHOD

The limit of quantification has not been defined. The lowest level at which the method has been validated is a fortification level of 0.004 ppm. Method validation data is presented in Appendix 2.