Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Primisulfuron-Methyl and Metabolites

MRID #: 420401-02

Matrix: Soil

Analysis: LC/MS/MS

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APPENDIX 1. Analytical Method CIGPSM1

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Method No.: CIGPSM1

Subject: Determination of Primisulfuron-Methyl (CGA-136872) and its Metabolites in Water and Soil by High Performance Liquid Chromatography Thermospray Mass Spectrometry (TSP-LC/MS)

Revision No.: 2 Effective Date: 6/16/91 Supersedes: Rev. 1 (Draft)

I. INTRODUCTION/SUMMARY

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A. Scope

This method is used for the determination of primisulfuron-methyl (CGA-136872, 3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl)-1-[2-methoxycarbonylphenyl sulfonyl] urea) and its metabolites CGA-191429 (2-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]aminocarbonyl-aminosulfonylbenzoic acid), CGA-120844 (methyl 2-[aminosulfonyl]benzoate), CGA-177288 (2-[aminosulfonyl]benzoic acid), CGA-171683 (4,6-bis[difluoromethoxy]-2-aminopyrimidine), and CGA-27913 (1,2-benzisothiazol-3(2H)-one,1,1-dioxide) in water and soil with high performance liquid chromatography/mass spectrometry (LC/MS). A thermospray interface is used to introduce the HPLC column effluent into a triple stage quadrupole mass spectrometer, (TSP-LC/MS/MS). The structures and chemical names of the compounds are presented in Figure 1. Five analytes are analyzed by selected ion monitoring in one run by either negative ion MS, negative ion MS/MS, or positive ion MS/MS. The sixth analyte (CGA-177288), is analyzed by negative ion MS/MS using a different analytical column and mobile phase composition. The approximate instrument detection limits and detection method used for each compound are summarized in Tables III and IV. Reporting limits of 0.05 ppb and 0.5 ppb are obtained for all compounds in water and soil, respectively. Method development work for the extraction and cleanup of the analytes from water and soil was conducted at CIBA-GEIGY. Method validation and all LC/MS work was conducted at ALTA.

This method is restricted to use under the supervision of analysts experienced in the operation of TSP-LC/MS/MS.

Prepared by: Robert A. Bethem

Management Approval: Robert A. Bethem Date: 9-9-91

QA Officer Approval: Robert S. Peterson Date: 9-9-91

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B. Principle

1. Water

A 100-mL aliquot of a water sample is made acidic with phosphoric acid and extracted with methylene chloride and then ethyl acetate. The organic extracts are evaporated to near dryness on a rotary evaporator and the residue is redissolved in 1.0-mL of acetonitrile. Just prior to analysis, a 200 ul sample aliquot is taken to near dryness and then re-diluted to 200 ul with 0.2M NH_4OAc. The extract is then analyzed for parent and four degradation products by high performance liquid chromatography/mass spectrometry (LC/MS). A second 100 ul aliquot is also taken, brought to complete dryness, re-diluted with 100 ul of 0.2M NH_4OAc, and analyzed for CGA-177288 by LC/MS on a different LC column in an acidic mobile phase. A flow chart for this process can be found in Figure 1 of this method.

2. Soil

Soil samples (20 g) are extracted at room temperature with mechanical shaking in 50-mL of 20% (v/v) methanol/phosphate buffer (0.03 M, pH adjusted to 6.0). After centrifugation, a 25-mL aliquot of the soil extract is diluted with water, acidified, and then extracted with methylene chloride and then with ethyl acetate. The organic extracts are evaporated to near dryness on a rotary evaporator and the residue is redissolved in 1.0-mL of acetonitrile (ACN). Just prior to analysis, a 200 ul sample aliquot is taken to near dryness and then re-diluted to 200 ul with 0.2M NH_4OAc. The extract is then analyzed by high performance liquid chromatography mass spectrometry (LC/MS). A second 100 ul aliquot is also taken, brought to complete dryness, re-diluted with 100 ul of 0.2M NH_4OAc, and analyzed by LC/MS with a different LC column in an acidic mobile phase. A flow chart for this process can be found in Figure 2 of this method.

C. Safety

The toxicity or carcinogenity of each reagent used in this method has not been precisely determined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of available material data handling sheets should be available to all personnel involved in the chemical analysis.

II. MATERIALS AND METHODS

A. Apparatus

1. Bottle, amber Boston round, appropriate size for storage of standard solutions.
2. Bottle, polypropylene, with cap, appropriate size for soil extractions.
3. Centrifuge, IEC Centra 8 (International Equipment Corporation) or equivalent.
4. Flask, round bottom, 500-mL.
5. Flask, Erlenmeyer, 250-mL.
6. Funnel, filter.
7. Funnel, separatory, 250-mL.
8. Glass wool (Fisher cat. #11-390) or equivalent.
9. Mechanical shaker, orbital (Fisher cat. #12-812) or equivalent.
10. pH stick, Corning (Fisher cat. #13-641-536) or equivalent.
11. Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent.
12. Tube, concentration/centrifuge, 50-mL (Fisher cat. #05-538-40B) or equivalent.
13. Syringe, 5-mL plastic, (BD #9603) or equivalent.
14. Filter, syringe, 0.45u (Gelman 4472 Acrodisc 3 CR PTFE) or equivalent.
15. Balance, Analytical, capable of weighing to the nearest 0.0001 g.
16. Vials, 1-mL (Waters 78514 or equivalent).
17. Vials, 4-mL (Waters 72710 or equivalent).
18. Low volume inserts (Waters 72704 or equivalent).
19. Caps with septa (Waters 73010 or equivalent).

B. Reagents

1. Acetone, Optima grade (Fisher cat. #A929-4) or equivalent.
2. Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.
3. Ethyl Acetate, HPLC grade (Fisher cat. #E195-4) or equivalent.
4. Methylene chloride, HPLC grade (Fisher cat. #D143-4) or equivalent.
5. Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.
6. Ammonium acetate, crystals, (EM Science cat. #AX1220-1) or equivalent.
7. Extraction solvent (soil): 20% (v/v) methanol/phosphate buffer.
8. HPLC mobile phase, 0.2M ammonium acetate. Dissolve 15.4 grams of ammonium acetate in 1 L of water.
9. Phosphate buffer, 0.03 M, pH = 6.0 ± 0.5. Dissolve 2.1 grams of sodium phosphate monobasic monohydrate in 500-mL of purified water. Adjust the pH to 6.0 ± 0.5 with phosphoric acid and sodium hydroxide.
10. Phosphoric acid, 85% (Conc.) (Fisher cat. #A242-1) or equivalent. 1% (v/v) Phosphoric acid/water.
11. Sodium hydroxide, reagent grade, 50% (w/w) (Fisher cat. #SS254-500) or equivalent.
12. Sodium phosphate monobasic, monohydrate, (Fisher cat. #S369-500) or equivalent.
13. Sodium sulfate, anhydrous (Fisher cat. #S421-3) or equivalent.
14. Water, distilled, HPLC grade, or purified in-house with a HYDRO™ purification system, or equivalent.
15. CGA-136872, CGA-191429, CGA-120844, CGA-177288, CGA-171683, and CGA-27913 analytical standards, CIBA-GEIGY Corp., P. O. Box 18300, Greensboro, NC 27419.

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C. Analytical Procedure

1. Water

1.1 Weigh a 100 g aliquot of the water sample into a 250-mL separatory funnel. (Note: A smaller aliquot may be used, but this will increase the limit of determination in ppb for the analyte. A larger aliquot may also be used to increase the sensitivity of the analysis). Alternatively, measure out in a graduated cylinder 100-mL of the water sample and transfer to a 250-mL separatory funnel.

1.2 Add 2.0-mL of conc. phosphoric acid and shake to mix. The pH of the aqueous solution should be < 2.5. Sample fortification, if required, should be done at this time (refer to Section II.J.2.0).

1.3 Add 75-mL of methylene chloride. Shake for thirty seconds. Allow the two phases to separate. A smaller volume of extracting solvent may be used if satisfactory recoveries are demonstrated in the field matrix spikes.

1.4 Place a small glass wool plug into a filter funnel. Add approximately 50 g of sodium sulfate, and rinse with approximately 10-mL of methylene chloride. Drain the lower organic layer through the sodium sulfate into a 500-mL round bottom flask.

1.5 Extract the sample with a second 75-mL aliquot of methylene chloride. Follow the directions in Step 1.3 and collect the organic phase into the same 500-mL flask. Rinse the sodium sulfate with an additional 10-mL of methylene chloride and drain into the 500-mL flask.

1.6 Add 75-mL of ethyl acetate. A smaller volume of extracting solvent may be used if satisfactory recoveries are demonstrated in the field matrix spikes. Shake for thirty seconds. Allow the two phases to separate. Drain the lower aqueous phase into a 250-mL erlenmeyer flask. Drain the organic phase through the sodium sulfate (pre-rinsed with approximately 10-mL of ethyl acetate) into the 500-mL flask containing the organic extracts from Steps 1.3 and 1.4.

1.7 Pour the aqueous portion back into the separatory funnel, (rinsing the erlenmeyer with ethyl acetate), and extract the aqueous sample with a second 75-mL aliquot of ethyl acetate. Follow the directions provided in Step 1.6 and collect the organic into the same 500-mL flask.

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1.8 Rinse the separatory funnel with ethyl acetate draining through the sodium sulfate into the 500-mL flask. Rinse the sodium sulfate three times with approximately 10-mL of ethyl acetate each. Repeat this procedure using methylene chloride.

1.9 Remove the organic solvents from the sample by placing the 500-mL flask on a rotary evaporator with a water bath temperature of approximately 35°C. Remove the flask when approximately 5-mL remain. Note: Do not allow the extract to go to dryness in any of the concentration steps.

1.10 Transfer the extract to a 50-mL concentration tube, rinsing the round bottom 5 times with approximately 3-mL each of acetone.

1.11 Add approximately 2-mL of acetonitrile to the concentration tube.

1.12 Place the tube on a rotary evaporator (water bath temperature of approximately 35°C) and remove the solvent until just under 2-mL remains.

1.13 Rinse the walls of the tube with approximately 6-mL of acetonitrile.

1.14 Place the tube on the rotary evaporator (water bath temperature of approximately 35°C) and remove the solvent until just under 1-mL remains. Remove the tube from the evaporator.

1.15 Adjust the volume to 1.0-mL with acetonitrile.

1.16 Transfer the contents to a five-mL syringe with a 0.45 micron filter and collect the filtered extract into a 1-mL vial.

1.17 Store the acetonitrile extract at less than -5 degrees C until the day of analysis.

1.18 On the day of analysis for all analytes except CGA-177288, aliquot 200 ul of the extract into a 1-mL autosampler vial and concentrate to near dryness (meniscus at bottom of the 1-mL vial) with nitrogen. Adjust the volume back to 200 ul with 0.2M NH_4OAc.

1.19 Analyze by LC/MS using the HPLC conditions in Table I and LC/MS conditions in Table III.

1.20 On the day of analysis for CGA-177288, aliquot 100 ul of the extract into a 1-mL autosampler vial and concentrate to complete dryness with nitrogen. Alternatively, a 100 ul aliquot may be taken to dryness after step 1.16 and stored according to 1.17. Adjust the volume back to 100 ul with 0.2M NH_4OAc.

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1.21 Analyze by LC/MS using the HPLC conditions in Table II and LC/MS conditions in Table IV.

2. Soil

2.1 Weigh 20 g of soil sample into a 250-mL polypropylene centrifuge bottle. Sample fortification, if required, should be done at this time (refer to Section II.I.2.0).

2.2 Add 50-mL of the soil extraction solvent. Place the cap on the bottle. Place the bottle in an orbital shaker and shake the sample for two hours at room temperature, or 25°C if the shaker has a water bath.

2.3 Remove the sample from the shaker. Place the bottle in the centrifuge at approximately 4000 rpm for approximately 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.4 Add 75-mL of water, 2.0-mL of conc. phosphoric acid, and 25-mL of the supernatant from Step 2.3 to a 250-mL separatory funnel. Shake the contents to mix. The pH of the aqueous solution should be < 2.5.

2.5 The remainder of the cleanup procedure is identical to the procedure for water. At this point refer to Step 1.3 above (the methylene chloride extraction) and follow Steps 1.3 through 1.21.

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D. Instrumentation

1. Description and Operating Conditions - HPLC

See Table I for a description of the HPLC system and chromatographic conditions for CGA-27913, CGA-191429, CGA-120844, CGA-136872, and CGA-171683. See Table II for a description of the HPLC system and chromatographic conditions for CGA-177288.

2. Description and Operating Conditions - Mass Spectrometer

In order to achieve maximum sensitivity and specificity for these analytes, it has been necessary to operate the instrument in both positive and negative ionization modes. With the exceptions of CGA-27913 and CGA-171683, all data is collected using MS/MS techniques. There are two primary reasons for employing MS/MS technology.

- Thermospray ionization is a "soft" ionization technique, much like chemical ionization, and usually results in only one ion for detection. MS/MS induces additional fragmentation on this ion (CID) and, therefore, provides additional mass spectral information for confirmation of analyte identity.

- Due to the high chemical specificity of the technique, detector noise is dramatically reduced and signal to noise is enhanced. This in turn leads to lower detection limits and greatly reduces the occurrence of interferences.

CGA-27913 and CGA-171683 do not yield adequate product ions for analysis by MS/MS.

Standard mass spectrometer source tuning techniques are used for thermospray mass spectrometry. Mass calibration is based on the most recent FC-43 calibration by electron impact ionization. Mass calibration may be checked in TSP mode by checking background ions at m/z 59, m/z 187, and m/z 269. Normally, no calibration adjustments are necessary.

When the instrument is operated in the product ion mode (MS/MS), quantitation is based on the area of the product ion resulting from the collision induced dissociation (CID) of the protonated molecular ion or ammonium adduct ion (precursors).

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During the course of the analytical run for primisulfuron-methyl and four of its degradation products, the instrument must be switched from negative ion (Q1MS), to positive ion MS/MS, and then back to negative ion (Q1MS). Switching times must be selected so that an adequate number of scans precede and follow each peak to be quantified. See Table III for a description of the scanning modes and switching times for the mass spectrometer.

The analysis for CGA-177288 is performed by negative ion MS/MS. See Table IV for a description of the thermospray and instrument operating conditions.

3. Description and Operating Conditions - Thermospray Interface

Extensive optimization studies were performed to determine the best operating parameters for each of the six analytes. In general, CGA-177288 and CGA-27913 give optimum response at high ion source temperatures, with the remaining compounds (especially CGA-120844) optimizing at cooler source temperatures. On the Finnigan TSP2 interface, a source temperature of 260° C. seems to provide the best results overall. The optimized values for the TSP vaporizer may vary with time. In general, the optimum vaporizer temperature is found to be approximately 15 degrees C. below the take off temperature as evidenced by the m/z 59 reagent ion. With the Finnigan TSP2 vaporizer with a sapphire tip, 100° C. is usually the best setting.

See Table III and Table IV for a description of the operating parameters for the thermospray interface and mass spectrometer.

4. Calibration and Standardization

4.1 Determine the retention time of the analytes by injecting a standard solution. During a series of analyses the retention time should vary no more than 2% from the mean value, on a daily basis.

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4.2 Calibrate the instrument by using the average response factor from detector response (chromatographic peak area) and the amount of analyte injected encompassing a range from 0.125 ng to 5 ng, (50-ul injections). If the standard deviation for the three point curve is less than 20%, then the method is considered to be linear. A broader concentration range for the three point curve may be used providing that the detector response remains linear.

4.3 Generally, analytes should be calibrated relative to the average response factor from the two preceding standards and one standard following. In any event, the three standards used must be of three different concentrations encompassing the range of calibration.

4.4 Sample screening for none detectable amounts may be employed without regards to the 20% RSD criteria, providing that the low standard (2.5 ng/mL) yields adequate signal to noise (greater than 5:1) for each analyte.

E. Interferences

1. There are no known interferences originating from the sample cleanup procedure.

2. Interferences have been observed for CGA-27913 at concentrations less than the reporting limit for soil and water.

F. Confirmatory Techniques

1. Although no alternative techniques exist, this method provides detection based on highly specific negative ion or MS/MS techniques. In addition, retention time data is also available for confirmation.

G. Time Required

1. The sample cleanup procedure can be completed for a set of eight samples in ten hours.

2. Each LC/MS analysis for parent and four degradation products requires 20 minutes.

3. Each LC/MS analysis for CGA-177288 requires 5 minutes.

H. Modifications and Potential Problems

1. Degradation of CGA-120844 is observed in aqueous solutions within a relatively short time (less than 72 hours). Therefore, extracts are stored in 100% acetonitrile in the freezer until the day of analysis. Prior to analysis, an aliquot of the extract is taken to near dryness and re-dissolved in 0.2M NH_4OAc.

2. Stock solutions must be prepared in 100% acetonitrile and stored in the freezer. LCMS analytical standards should be prepared in the initial mobile phase from the stock solution every 72 hours or sooner.

3. CGA-171683 will only partition into methylene chloride. CGA-177288 and CGA-27913 do not partition quantitatively into methylene chloride and require the additional ethyl acetate extractions.

4. Due to the volatility of CGA-171683, close supervision of samples is required during concentration steps. The evaporation techniques used for the sample aliquots prior to analysis is critical. Autosampler vials (1-mL) are used for both analyses. For CGA-177288, the 100 ul aliquot must be taken to complete dryness in order to avoid signal enhancement during analysis. For the parent analysis, the 200 ul sample aliquot must not be taken down beyond 20 ul or the recovery for CGA-171683 will drop below 50%. In a 1-mL vial, 20 ul remains when the meniscus just touches the bottom of the vial.

5. The suggested operating temperatures were developed for a Finnigan TSP2 source as a result of extensive optimization studies. In general, those analytes run by negative ion optimize at "hot" source temperatures, and those run by positive ion optimize at "cool" temperatures. The suggested temperatures are therefore set at an intermediate value.

6. Enhancement of signal has been observed for CGA-120844, CGA-177288, and to a much lesser degree, the parent compound. For CGA-177288, taking the extract to complete dryness and injecting into an acidic mobile phase has corrected the problem for most analyses. Recoveries for CGA-120844 were observed to be as high as 151% during water method validation.

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I. Preparation of Standard Solutions

1. A 1 mg/mL stock solution for each analyte is prepared by dissolving 25.0 mg of pure material into 25-mL of acetonitrile. If the compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Transfer the stock standard solution into an amber bottle and seal with teflon lined caps only. Store the standards at -10°C or colder and protect from light when not in use. Stock standard solutions must be replaced after 1 year or sooner if comparison with check standards indicates a problem.

2. Using the 1 mg/mL stock solutions from Step 1, prepare a 10 ug/mL mixed standard containing all six compounds in acetonitrile. Replace the analytical solution every six months or sooner.

3. Fortification standards are prepared by dilution of the stock solution (Step 1.0) with acetonitrile. Fortification standards should be prepared such that no more than 2-mL of the solution is added to a sample. (Example: for a 100-mL water sample, the addition of 1.0-mL of a 0.5 ng/μL standard results in a fortification level of 5.0 ppb.)

4. LCMS analytical standards must be made fresh every 72 hours or sooner in the initial HPLC mobile phase. Standards should be prepared such that at least one is lower than the desired screening level and one is higher than the highest expected amount. Typically, a 10 ug/mL (ACN) stock solution is used for making the daily injection standards. (Example: for a 100 ng/mL standard, 100 ul of the stock solution is diluted to 10-mL in the initial mobile phase. Serial dilutions of 1:10, and 1:4 are then made for the 10 ng/mL and 2.5 ng/mL standards. Injection standards and fortification standards should be traceable to the same stock solution. Some record of the date that injection standards were diluted into the aqueous mobile phase must be maintained. If possible, this notation may be made directly into each standard data file header.

J. Determination of Sample Residues

1. Samples

1.1 Inject the sample solution from Step II.C.1.18 into the HPLC. The sample solution may be diluted with the sample diluent if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (ng/ml) is determined by multiplying the value of the chromatographic peak area by the average response factor (II.D.4.2).

2. Fortified Samples

The method is validated for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 0.05 ppb or more of each analyte (water) or with 0.5 ppb or more of each analyte in soil.

2.1 Add an appropriate volume of a fortification solution (from Step II.I.3) to 100-mL of a water sample prior to any of the cleanup steps. (Note: A sample size smaller than 100-mL may be used, but this will increase the determination limit in ppb.) The total volume of the added fortification solution should not exceed 2.0-mL (example: add 1.0-mL of a 0.5 ng/μL fortification solution to 100-mL of a water sample to fortify at 5.0 ppb.) For soil samples, fortify 20 g of the sample and allow sufficient time for the fortification solvent to evaporate before proceeding to Step 2.2

2.2 Proceed with the sample cleanup procedure.

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3. Calculations

3.1 Calculate the response factor (in ng/ml) from equation (1):

$$RF(ng/ml) = \frac{Conc_{STD}}{Area_{STD}}$$

Where: $Area_{STD}$ = the area response from a standard injection

$Conc_{STD}$ = concentration of the injected standard (ng/ml)

3.2 Quantitation of samples will be performed based on the average response for the standards immediately preceding and following the samples and is defined as follows:

$$RF_{AVG} = \frac{(RF_{STD1}) + (RF_{STD2}) + (RF_{STD3})}{3}$$

Where: RF_{STD1} = the response factor from the first standard

RF_{STD2} = the response factor from the second standard

RF_{STD3} = the response factor from the third standard

Normally, RF_{STD1} and RF_{STD2} will precede the sample and RF_{STD3} will follow.

The per cent relative standard deviation for RF_{AVG} must be less than 20%. If the instrument response falls outside of this range after two re-injections of the standard, then the affected samples must be re-injected. In the instance of screening samples where no positive detections are made, it is only necessary to demonstrate adequate system sensitivity via injection of the low calibration standard.

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3.3 Sample calculations are done according to the following formula:

$$Amount_{SAMP} = \frac{(Area_{SAMP})(RF_{AVG})(FV)}{(Wt_{SAMP})\ (Df)}$$

And,

3.4

$$Amt_{SAMP} = \frac{(Area_{SAMP})(RF_{AVG})}{(Cf)\ (Df)}$$

Where: Amt_{SAMP} = Final Sample Amount (ppb),

$Area_{SAMP}$ = Area response from unknown,

RF_{AVG} = Response Factor (ng/ml),

FV = Final Volume (ml),

Wt_{SAMP} = Sample weight (g),

Cf = Concentration factor (Wt_{SAMP}/FV),

Df = Dilution factor (Vol_{inj}/Vol_{inj}).

3.5 If the sample amount calculated from the above equation is greater than the screening level for soils or waters, then one or more of the following additional calculations are made:

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3.6 Soil or water residue values are corrected for recoveries less than 100% according to the following formula:

$$Amount_{REC} = \frac{Amount_{SAMP}\ (ppb)}{\frac{R\ (\%)}{100}}$$

3.7 Soil residue values are corrected for recoveries less than 100 % and/or percent moisture:

$$Amount_{DryWt} = \frac{Amount_{SAMP}\ (ppb)}{(M)}$$

$$Amt_{DryWt,\ REC} = \frac{Amt_{SAMP}\ (ppb)}{(\frac{R\ (\%)}{100})\ (M)}$$

Where: Amt_{DryWt} = Final Sample Amount percent moisture corrected

Amt_{REC} = Recovery corrected for Sample Amount

$Amt_{DryWt,REC}$ = Final Sample Amount corrected for average recoveries and percent moisture,

R = Average Recovery of fortified control sample(s) where R is less than 100 %,

M = Moisture content correction factor (DryWt/WetWt).

3.8 If $Amount_{SAMP}$ is less than the reporting limit then the results are reported as less than this amount and all other calculations are reported as "NC" (not calculated). Field sample results greater than twice the screening limit are reported to two significant figures. All other field sample results exceeding the screening limit are reported to one significant figure.

3.9 The results from fortified control samples are reported to three significant figures if the spike level is greater than twice the screening limit. All other spike results are reported to two significant figures. Percent recoveries are reported to two significant figures if the result is less than 100% and three significant figures if the result is greater than 100%.

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TABLE I. HPLC OPERATING CONDITIONS FOR THE DETERMINATION OF PRIMISULFURON-METHYL (CGA-136872) AND FOUR METABOLITES BY TSP-LC/MS/MS

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent)

Waters 590-MS HPLC isocratic pump (or equivalent)

Waters WISP 712 autosampler (or equivalent)

Operating Conditions:

Column:	Nova-Pak, 4 um, 4.0 mm x 15 mm HPLC column, Waters part # 86344
Mobile Phase:	10% (v/v) acetonitrile/(water with 0.2M NH_4OAc), step gradient to 30/70 at 0.1 minute, step gradient to 55/45 at 3.5 minutes, step gradient to 100% ACN at 8 minutes, re-equilibrate at 12 minutes.
Flow Rate:	1.2 ml/min
Injection Volume:	50 ul
Cycle Time:	20 minutes

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TABLE II. HPLC OPERATING CONDITIONS FOR THE DETERMINATION OF CGA-177288 BY TSP-LC/MS/MS

Instrumentation:

Waters 600-MS HPLC gradient pump (or equivalent)

Waters 590-MS HPLC isocratic pump (or equivalent)

WISP 712 autosampler (or equivalent)

Operating Conditions:

Column:	Two Zorbax Rx-C8 Reliance columns, (4mm x 80 mm 5 micron), (MAC MOD part # 820967-901) in series using fitting kit (MAC MOD part # 820678-901).
Mobile Phase:	Isocratic at 3% (v/v) acetonitrile/(1% HOAc with 0.2M NH_4OAc).
Flow Rate:	1.2 ml/min
Injection Volume:	50 ul
Cycle Time:	5 minutes

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TABLE III. TSP-LC/MS/MS OPERATING CONDITIONS FOR THE DETERMINATION OF PRIMISULFURON-METHYL (CGA-136872) AND FOUR METABOLITES BY TSP-LC/MS/MS

Instrumentation:

Mass Spectrometer: Finnigan MAT TSQ-700 capable of positive and negative ion MS and/or MS/MS and equipped with a TSP2 thermospray interface (or equivalent).

Operating Parameters:

Collision gas pressure: 2.5 mtorr (2.0 - 3.0 mtorr)

Collision offset: -18 volts (-10 - -20 volts, positive ion)
18 volts (10 - 20 volts, negative ion)

Vaporizer Temperature: 100 degrees C (90 - 110 degrees C)

Source Temperature: 260 degrees C (220 - 300 degrees C)

Repeller Voltage: 40 volts (0 - 50 volts, positive)
-20 volts (0 - -50 volts, negative)

Analyte	MW	Analysis Mode	Ions monitored (precursor ions)	Retention Time (min.)	Detection Limit
CGA-136872	468	MS/MS (+) 3 - 7.1 min.	(271) - 160 (233) - 199	6:30	10 pg
CGA-191429	454	MS/MS (+) 3 - 7.1 min.	(271) - 160	3:50	25 pg
CGA-120844	215	MS/MS (+) 3 - 7.1 min.	(233) - 199	4:00	10 pg
CGA-27913[1]	183	MS (-) 1 - 3 min.	182	1:40	25 pg
CGA-171683[1]	227	MS (-) 7.1 - 10 min.	226	8:40	25 pg

[1] This analyte is scanned in the Q1MS mode with the collision gas on.

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TABLE IV. TSP-LC/MS/MS OPERATING CONDITIONS FOR THE DETERMINATION OF CGA-177288 BY TSP-LC/MS/MS

Instrumentation:

Mass Spectrometer: Finnigan MAT TSQ-700 capable of negative ion MS/MS and equipped with a TSP2 thermospray interface (or equivalent).

Operating Parameters:

Collision gas pressure:	2.5 mtorr (2.0 - 3.0 mtorr)
Collision offset	18 volts (10 - 20 volts)
Vaporizer Temperature:	100 degrees C (90 - 110 degrees C)
Source Temperature:	260 degrees C (220 - 300 degrees C)
Repeller Voltage:	-20 volts (0 - -50 volts)

Analyte	MW	Analysis Mode	Ions monitored (precursor ions)	Retention Time (min.)	Detection Limit
CGA-177288	201	MS/MS (-) 1.3 - 3.2 min.	(200) - 92	2	25 pg

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FIGURE 1.

FLOW DIAGRAM FOR THE DETERMINATION OF CGA-136872 AND METABOLITES IN WATER

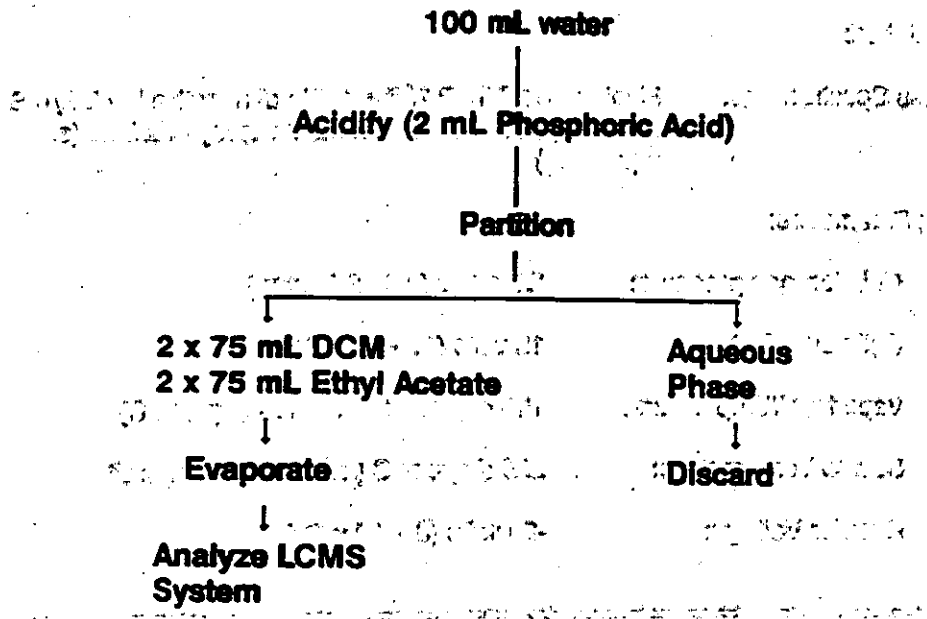

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FIGURE 2.

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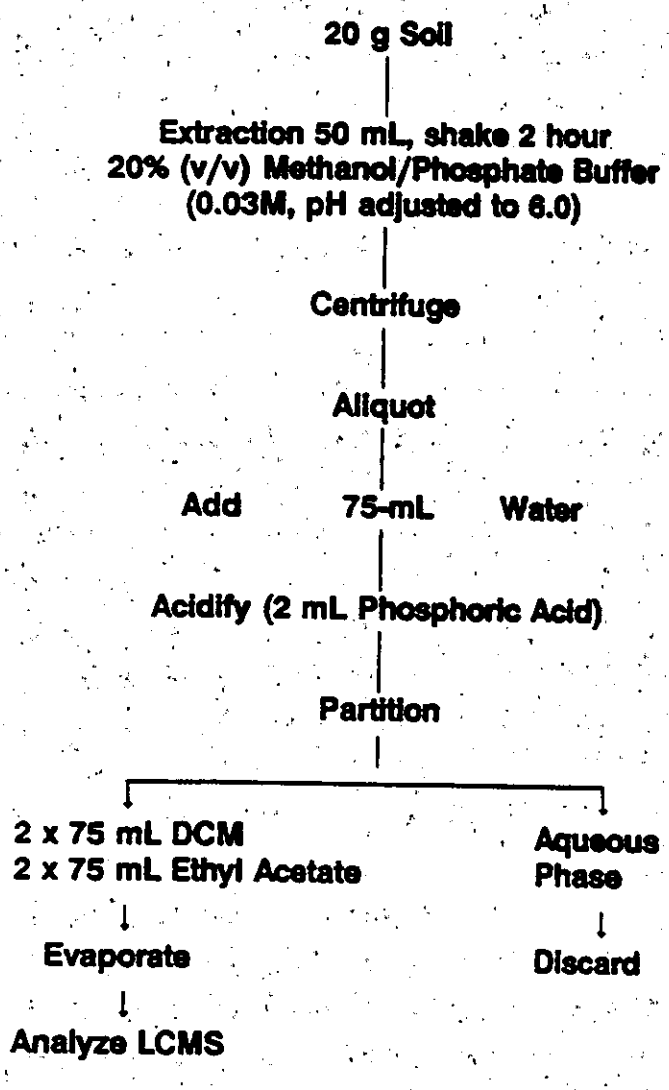
FLOW DIAGRAM FOR THE DETERMINATION OF CGA-136872 AND METABOLITES IN SOIL



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APPENDIX 2. Analytical Spreadsheets

A. Water Method Validation Study I
B. Water Method Validation Study II
C. Soil Method Validation Study I