1.0 INTRODUCTION

This method provides a means of determining ametryn and selected metabolites as residue in soils. Validation levels of metabolites ranged from 0.005 ppm to 0.50 ppm while ametryn was validated from 0.005 ppm to 5.0 ppm. Recoveries were greater than 79% for all compounds with no background levels detected for any compound. Minimum quantitation level is set at 0.01 ppm for all compounds.

2.0 PRINCIPAL OF METHOD

Ametryn and metabolites are extracted from soil using 80% methanol: 20% water in a soxhlet extractor. After 10-14 hours of extraction the extraction fluid in cooled, then brought to a volume of not less than 200 ml and split evenly. One half the extract is placed into a 500 ml separatory funnel, and reserved for hexane partitioning to extract for ametryn.

The other half of the extract is rotary evaporated to 1-2 ml and cleaned up by strong cation exchange column chromatography. The cleaned up extract is then chromatographed using two HPLC conditions to quantitate the three metabolites.

The hexane partition is performed by addition of 100 ml of 1% aqueous NaCl to the extract and two partitioning of hexane. After removal of the hexane, the extracts are reconstituted in methanol and quantitated using gas chromatography with nitrogen-phosphorous detection.

3.0 REAGENTS

80% MeOH:20% H_2O - for each liter required combine 800 ml of HPLC grade methanol and 200 ml of reagent grade (millipore) water.

1% NaCl aqueous - for each liter required combine 10 g NaCl and 1 liter of reagent grade (millipore) water.

25% MeOH:74% H_2O:1% acetic acid - into a 500 ml graduated container, place 5 ml of acetic acid and 125 ml of HPLC grade methanol. Bring this container to a volume of 500 ml using reagent grade water and mix.

1% acetic acid aqueous - for each liter required combine 10 ml of glacial acetic acid with 990 ml of reagent grade water.

0.1 M KH_2PO_4 (monobasic potassium phosphate) for each 100 ml of reagent combine 1.36 g KH_2PO_4 with 100 ml of reagent grade water.

80% 0.3 M K_2HPO_4:20% MeOH - for each 500 ml of reagent combine 20.9 of K_2HPO_4 (potassium phosphate dibasic) with 400 ml of reagent grade water. Dissolve the K_2HPO_4 in the water prior to adding 100 ml of HPLC grade methanol.

Aqueous mobile phase - for each liter of aqueous mobile phase weigh 4.08 g of KH_2PO_4 and 4.00 g of octane sulfonic acid sodium salt and dissolve in 1 liter of reagent grade water. Filter through a 0.45 µ filter.

60% CH_3CN mobile phase - for each liter of 60% CH_3CN mobile phase weigh 4.08 g of KH_2PO_4 and 4.00 g of octane sulfonic acid sodium salt and dissolve in 400 ml of reagent grade water. After dissolving the solids in the water, add 600 ml of acetonitrile. Filter through a 0.45 µ filter.

4.0 STANDARD PREPARATION

Individual standards of ametryn and metabolites are prepared in separate solutions and then diluted or combined and diluted to form mixed standards.

Individual standards are weighed, corrected for purity, as specified below into methanol.

Compound	Weight (mg)	Volume (ml)	Concentration µg/ml
Ametryn	20.0	100	200
GS-11354	20.0	100	200
G-34048	12.5	250	50.0
GS-17794	12.5	250	50.0

Solubility of these compounds in methanol is marginal at the indicated concentrations. Particularly, G-34048 and GS-17794 are difficult to dissolve at room temperature and will precipitate when taken to freezer temperature.

Before using any of the standards they must be brought to room temperature and shaken or placed in an ultrasonic bath.

Individual spiking solutions are prepared in methanol as indicated.

Compound	Aliquot Volume (ml)	Dilution Volume (ml)	Concentration µg/ml
Ametryn	5	100	10
GS-11354	5	100	10
GS-17794	20	100	10
G-34048	20	100	10

A mixed standard is also prepared using the scheme indicated above and combining all components into a single volumetric flask. This provides a single solution containing 10 µg/ml of each component.

Typical spiking levels used during the conduct of the ametryn study are listed:

Indicated Spike	Compound	Concentration µg/ml	Volume Used ml	Spike Level ppm
AMT 5.0	Ametryn only	200	2.5	5.0
MXD 0.5	All components	10	5.0	0.5
MXD 0.05	All components	10	0.5	0.05
MXD 0.01	All components	10	0.1	0.01

Gas chromatography standards are made by dilution of the ametryn only standard as indicated in methanol.

Standard Level (ng/ml)	Stock Concentration (µg/ml)	Aliquot Volume (ml)	Dilution Volume (ml)
1000	10	10	100
500	10	5	100
250	1	25	100
50	1	5	100
25	0.25	10	100

These dilutions are given as examples. Other combinations of dilutions should work equally well so long as the concentration range remains essentially intact.

HPLC standards are prepared by dilution of the 10 µg/ml mixed spiking solution as indicated.

Concentration ng/ml	Percent Solution Concentration	Aliquot Volume (ml)	Dilution Volume (ml)	Dilution Fluid
500	10 µg/ml	5	100	10 ml of acetonitrile and 85 ml aqueous mobile phase buffer.
250	500 ng/ml	50	100	15% acetonitrile in aqueous mobile phase buffer.
50	250 ng/ml	20	100	15% acetonitrile in aqueous mobile phase buffer.
25	50 ng/ml	50	100	15% acetonitrile in aqueous mobile phase buffer.

Other dilution schemes would work equally well. The standard curve level should remain essentially intact and the injection

fluid must be made to contain aqueous mobile phase buffer and not more than the 15% organic, acetonitrile or methanol in combination.

5.0 EXTRACTION PROCEDURE FOR EVIK AND METABOLITES

1. Weigh 100 g of soil ±1 g into a 43 X 123 mm soxhlet extraction thimble.

2. Place 250 ml of 80% MeOH:20% H_2O into each 500 ml flat bottom. Place 3-10 boiling chips into each flat bottom.

3. Put the soxhlet extractor on top of the 500 ml flat bottom and place the soil sample contained in the extraction thimble into the extractor.

4. If required soil samples may be spiked by pipeting the proper amount and concentration of spiking solution directly onto the soil at this point.

5. The extraction apparatus is then placed onto the heating mantle and extracted over night (10-14 hours).

6. After extraction, the apparatus is allowed to cool and all the extraction solvent is drained into the 500 ml flat bottom. This is done by removing the condensor and tilting the extractor to allow the extraction solution to siphon. Note that some extraction solution may remain in the soxhlet.

7. The extract in the 500 ml flat bottom is then transferred into a 250 ml mixing cylinder. The extract is brought to a 200 ml volume if necessary with 80% MeOH:20% H_2O. One half the volume is returned to the 500 ml flat bottom which originally contained the extract. The other half is reserved for hexane partitioning.

8. The half of the extract that is returned to the 500 ml flat bottom is then taken to a 1 - 2 ml volume using vacuum rotary evaporation. A water bath temperature of 35 - 40°C can be used to remove the extraction solvent.

9. A volume of 8 ml of 25% MeOH:74% H_2O: 1% acetic acid is used to transfer the concentrated material from the flat bottom into a 15 X 125 mm culture tube. This transfer of material is best accomplished by adding an initial 2 ml of solvent mixture and rinsing the sides of the 500 ml flat bottom. The rinse solution is then transferred to the culture tube. The rinsing process is then repeated with 2 more 3 ml portion of the original 8 ml of rinse solvent.

10. This portion of the extract is then reserved for SCX column clean up.

6.0 STRONG CATION EXCHANGE (SCX) COLUMN CLEAN UP

1. Analytichem 3 cc. SCX columns (part # 617303) are packed firmly into a bed by tamping on the frit with a pasteur pipet.

2. The columns are then conditioned using 4 ml of MeOH then 4 ml of 1% acetic acid.

Note: During the conditioning process and all further steps, column flow rates should not exceed 1 drop per second and should not be allowed to go completely dry.

3. The reconstituted sample extract is then applied to the column.

4. The column is then washed with 2 ml of 1% acetic acid in water.

5. Then the column is washed with 2 ml of methanol.

6. Finally the column is washed with 2 ml of 0.1 M KH_2PO_4 (monobasic potassium phosphate).

7. The column is then eluted with 9 ml of 80% 0.3 M KH_2PO_4 (dibasic potassium phosphate):20% methanol into a graduated 10 ml receiver tube. This fraction contains the compounds of interest. At this point allow the columns to go to dryness.

8. Add 500 µl of glacial acetic acid to the elution solvent, then bring to a 10 ml total volume with 1% acetic acid in water. This is then mixed and injected into the HPLC to screen for metabolites of ametryn.

9. Dilution of the cleaned up extract is accomplished by using 15% acetonitrile:85% mobile phase buffer.

7.0 HEXANE PARTITIONING

1. The portion of the methanolic extract that was reserved for hexane partitioning is transferred to a 500 ml separator.

2. One hundred ml of 1% NaCl aqueous and 100 ml of hexane is added to each separator.

3. The separatory funnels are then shaken for one minute and the phases allowed to separate.

4. The lower portion of the separatory funnel is returned to the 250 ml mixing cylinder and saved.

5. The hexane extract is then dried by passing it through 1" of anhydrous Na_2SO_4 contained in a powder funnel.

6. The aqueous portion is returned to the 500 ml separator and partitioned again with 100 ml of hexane.

7. The aqueous portion is discarded and the hexane portion is dried by passing it through the same powder funnel into the same 500 ml flat bottom.

8. The anhydrous Na_2SO_4 is then washed with 20 ml of methylene chloride.

9. The hexane extract is then taken to dryness using rotary vacuum evaporation and a water bath at less than 30°C.

10. The dried extract is then reconstituted to 10 ml with methanol.

8.0 GAS CHROMATOGRAPHY

The parent ametryn is determined by gas chromatography with nitrogen-phosphorous detection. Typical operating conditions are given.

Column - 15 M X 0.53 mm of a 1.5 µ film thickness of DB-1 (J&W catalog #125-1012)

Oven Temperature - 180°C for 6 minutes then raised to 250°C at 30°C/minute and held for 1 minute

Injector Temperature - 250°C

Detector Temperature - 330°C

Column Flow - 7 to 10 ml/min He

Make Up Flow - 20 ml/min He

Detection Flow - 100 ml/min air
4 ml/min H_2

Injection volume - 1-2 µl

Conditions given allow ametryn to elute in 4.5 - 5.5 minutes which will allow resolution from GS-11354 which elutes at 3-4 minutes when present. See examples of gas chromatography attached. Example 1 shows ametryn and GS-11354 in the same chromatogram. Examples 2 through 5 show a high ametryn standard, a low ametryn standard, a processed control soil, and a low level processed spiked soil. GS-11354 did not partition favorably with hexane so gas chromatography is not used to quantitate it.

9.0 HPLC CHROMATOGRAPHY

Two isocratic HPLC conditions are used to quantitate the three selected metabolites of ametryn. The isocratic conditions used, utilize a wash off cycle at the end of the isocratic analytical period with a period of column equilibration before sample or standard injection.

Common HPLC system components and parameters used are given.

HPLC system - a system capable of delivering an isocratic mobile phase, then switching to a higher concentration of acetonitrile to wash the column, then equilibrating to the original isocratic condition is used. The system currently employed is a binary gradient system.

HPLC column - Supelco 15 cm LC-8DB, 5µ Catalog #5-8347

Detector - UV at 240 nm

Injection volume - 100 µl

HPLC pump flow rate - 2.0 ml/min

HPLC Conditions for GS-17794 - The isocratic mobile phase used for GS-17794 is 25% of the 60% acetronitrile:buffer mobile phase with the balance being aqueous mobile phase buffer for 10 minutes. The system is then changed to be 100% of the 60% acetonitrile: buffer over a 1 minute period and held for 4 minutes. The system is then returned to the original 25% of the 60% acetonitrile: buffer mobile phase with the balance being aqueous mobile phase buffer in 1 minute and being held for 6 minutes to equilibrate before the next injection period starts. This gives a retention time of 7-8 minutes for GS-17794. Examples 6 through 9 are chromatograms of a high standard, a low standard, an extracted soil control, and an extracted low level spike.

HPLC conditions for GS-11354 and G-34048 - The isocratic mobile phase used for determination of GS-11354 and G-34048 is 42% of the 60% acetronitrile:buffer mobile phase with the balance being aqueous mobile phase buffer for 7 minutes. The system is then changed to 100% of 60% acetonitrile:buffer mobile phase during a 2 minute period at 100% of the 60% acetonitrile:buffer mobile phase. The system is then changed back to the original 42% of the 60% acetonitrile:buffer mobile phase with the balance being aqueous mobile phase buffer during a 1 minute period and the equilibration period is maintained for 5 minutes before the next injection period starts. This gives typical retention times of 3.5 to 4.4 minutes for GS-11354 and 4.8 to 5.8 minutes for GS-34048. Example 10 shows chromatography of an extended period of the isocratic analytical period noted above. This example indicates ametryn eluting at about 18 minutes. This was not used for assay of this compound due to low recoveries through the SCX column, but is included for informative purposes. Examples 11 through 14 are examples of chromatograms of a high standard, a low standard, an extracted control soil and an extracted low level spike using conditions described for GS-11354 and G-34048.

10.0 CALCULATIONS

Concentrations of each component will be interpolated from a standard curve which brackets each sample set. If a response is above that of the highest standard then the sample solution will be diluted to be within the standard curve limits.

COUNT 11-13-90

~~ABC LABS #036153~~ pg 0091

ABC LABS #037895 pg 0201

Regression analysis and interpolating will be accomplished using the Beckman CALS® system. This system allows for data acquisition, data analysis, results reporting and information management.

By entering the weight of the sample processed and the extract volume into the CALS system a calculation of ppb residue is accomplished:

$$\text{ppb residue} = \frac{\text{ng/ml found X extract volume (ml)}}{\text{sample weight (g)}}$$

The ng/ml found in the interpolated value determined by the CALS program. The extract volume is entered into the CALS system as Std (RRF). This volume is calculated by:

$$\text{extract volume} = \frac{\text{Initial extract volume (ml) X dilution volume (ml)}}{\text{aliquot volume (ml)}}$$

The weight of the sample processed is entered into the CALS system as Smp (RRT).

Recovery of fortified control samples is calculated by:

$$\text{\% Rec.} = \frac{\text{Found ppm (fortified) - Found ppm *(control) X 100}}{\text{ppm added to fortified samples}}$$

*This value is calculated from the standard curve even if the level is below the lowest standard curve point. Values less than zero are not used in this calculation.

The average recovery of the fortified control samples is then divided into that residue level which was found to correct for method performance. This is the corrected ppm:

$$\text{Corrected ppm} = \frac{\text{ppm found X 100}}{\text{Average recovery of the residue}}$$

Moisture in analytical samples is determined as in ABC SOP F.C.1.7.1 (Soil Moisture Determinations). The calculations are also given in that SOP. Briefly the wet soil and containers are weighed and then dried to a constant weight at 105-130°C. The dry soil and containers are weighed after attaining a constant weight, usually overnight. Calculations are given:

$$\text{\% Moisture} = \frac{B - C}{B - A} \text{ X } 100$$

A = weight of container (g)
B = weight of wet soil and container (g)
C = weight of dry soil and container (g)

JELMT 11-13-90 ~~ABC LABS #A36153~~ A&C LABS #C 37595 pg 0892 pg C-3C 2C

The sample which contained any residues are then corrected to a dry matter basis:

$$\text{ppm dry} = \frac{\text{corrected ppm X 100}}{\text{100 - \% soil moisture}}$$

The molar equivalence is also determined and summed as follows to find ppm residue dry basis expressed as ametryn.

ppm dry expressed as =
Ametryn

(dry ppm GS-17794 X 1.34) + (dry ppm GS-11354 X 1.14)
(+ dry ppm G-34048 X 1.15) + dry ppm ametryn

ABC Lab Form No. 155

Addendum to Analytical Method

Method Sponsor: Ciba-Geigy Corporation

Method Number (if applicable): Amit 0589 ABC Lab# 37541, 37895, 38193, 38194

Method Title: Determination of Ametryne, GS-11354, G-34048 and GS-17794 in Soil

Effective Date: November 21, 1989

I certify that the following amendment to the above analytical method is to be made:

(1) Section 4.c. Standard Preparation. Gas Chromatography standards are to be made in Toluene in place of Methanol.

(2) Section 7.16 - Herbage Particulate - The dried extract is to be reconstituted to 10 mL in Toluene in place of Methanol

Brian Jungbauer
Study Director

11/29/89
Date

Attach this form to all laboratory and file copies of the applicable method.