The Determination of Fluvalinate and the Metabolites, Haloaniline, Chloroanilino Acid, and Phenoxybenzoic Acid.

1.0 SUMMARY

An analytical method for the measurement of fluvalinate and the metabolites, haloaniline, chloroanilino acid, and phenoxybenzoic acid in soil was developed by Chemalysis using excerpts from method numbers, AM-0812 (2/88) and IITRI CO8056-F (2/91). A fifty gram soil sample was extracted with methanol followed by a second extraction with 1:1 methanol:water. An aliquot was then mixed with 1 % $NaHCO_3$ and partitioned into methylene chloride. The organic phase was concentrated and exchanged into toluene for gas chromatograph (ECD) analysis of fluvalinate and haloaniline. The aqueous phase was adjusted to pH 12, heated to 65 C, cooled to room temperature, acidified to pH < 1, and partitioned into methylene chloride. The methylene chloride extract was concentrated and exchanged into mobil phase for HPLC (UV 205) analysis of chloroanilino acid and phenoxybenzoic acid.

2.0 ACCURACY AND PRECISION

The method was validated by fortifying three depths, 0 to 10 cm, 10 to 20 cm and 20 to 30 cm, of California control soil (Sandoz Crop Protection) at two levels, 0.01 ppm and 0.10 ppm in duplicate, with all four analytes. The results are summarized in Table 1.

3.0 SAFETY

The safety procedures outlined in Appendix I for all reagents and equipment used in this method are to be followed.

4.0 REAGENTS

4.1 Acetonitrile UV, Pesticide Grade, Burdick-Jackson

4.2 Methylene Chloride, Pesticide Grade, Burdick-Jackson

4.3 Ethyl Ether, non-preserved

4.4 Methanol, Pesticide Grade, Burdick-Jackson

4.5 Toluene, Pesticide Grade, Burdick-Jackson

4.6 Water, HPLC UV grade, Burdick-Jackson

4.7 Hydrochloric Acid, 37%, Mallinckrodt

4.8 Phosphoric acid, concentrated 85%

4.9 Potassium Hydroxide, analytical reagent grade, Mallinckrodt

4.10 Sodium Acetate Trihydrate, U.S.P.

4.11 Sodium Bicarbonate, reagent grade, Mallinkrodt

4.12 Sodium Sulfate, anhydrous, certified A.C.S., Fisher

5.0 STANDARDS

5.1 Fluvalinate, RS-FLU-101990, 92.4 % purity

5.2 Haloaniline, RS-HALO-11490, 99.8 % purity

5.3 Chloroanilino acid (CAA), RS-CAA-081689-2, 99.7 % purity

5.4 Phenoxybenzoic acid (PBA), RS-PBA-032890, 99.9 % purity

6.0 APPARATUS

6.1 Laboratory Equipment

6.1.1 Rotory Evaporator/Water Bath, Buchi 461

6.1.2 Centrifuge, International Equipment Company, Model PR-6

6.1.3 Platform Shaker, Eberbach, Ann Arbor, Michigan

6.2 Laboratory Glassware

6.2.1 Teflon Separatory Funnels, 1000 ml

6.2.2 Flat Bottom Flasks, 500 ml

6.2.3 Glass Filter Funnels

6.2.4 Teflon Wool (BITT Fiber), Alltech Asociates (stock #4082)

6.2.5 Polyethylene Bottles (8 oz), screw cap

6.2.6 Graduated Concentrator/Centrifuge tubes, 15 and 50 ml

6.2.7 Glass Volumetric Flasks, 50 ml, 100 ml

6.3 Analytical Instrumentation

6.3.1 Gas Chromatograph with Electron Capture Detector Hewlett-Packard 5890 Series II

6.3.2 Integrator, Hewlett-Packard model 3396

6.3.3 Liquid Auto-Sampler, Hewlett-Packard 7673A

6.3.4 Capillary Column, Restek RTX-5, 30 M x 0.53 mm, 1.0 um Film (bonded 95% dimethyl/5% diphenyl polysiloxane)

6.3.5 GC Temperatures:

Oven - 100 to 300 ° C program
100 ° C initial (time 0 min)
10 ° C/min to 150 ° C
30 ° C/min to 300 ° C
300 ° C Final (time 8 min)

Injector - 280 ° C

Detector - 325 ° C

6.3.6 Gases and Flow Rates:

Carrier - He @ 12 ml/min (10 psi)
Make-up - 95% Argon/5% Methane @ 30 ml/min

6.3.7 Injection - 2 ul Splitless (0.75 min)

6.3.8 GC Retention Times:

Fluvalinate - 14.4 min
Haloaniline - 3.6 min

6.3.9 HPLC, Waters 484 Tunable Absorbance Detector @ 205 nm (Sensitivity 0.02, Filter 1.0)

6.3.10 Waters pump, model 510

6.3.11 Waters, model 712 WISP auto-sampler

6.3.12 Integrator, Hewlett-Packard model 3396

6.3.13 Column, Supelco LC-18, 25 cm x 3.9 mm (5u)

6.3.14 Mobile Phase: 1:1:1 Methanol:ACN:Buffer
Buffer: 11 mM H3PO4/10 mM NaOAc

6.3.15 Flow Rate: 1.4 ml/min

6.3.16 Injection: 25 ul

6.3.17 HPLC Retention Times (flow 1.4 ml/min):

Chloroanilino Acid - 11.6 min
Phenoxybenzoic Acid - 5.6 min

7.0 PROCEDURE

7.1 Preparation of Standards

Stock Standard Solutions:
The following stock solutions were prepared using the analytical standards described in section 5.0.

7.1.1 Fluvalinate Stock - dissolve 108.4 mg standard into acetonitrile using 100 ml volumetric flask to yield a 1.0 mg/ml stock concentration.

7.1.2 Haloaniline Stock - dissolve 50 mg standard into methanol using a 50 ml volumetric flask to yield a 1.0 mg/ml stock concentration.

7.1.3 Chloroanilino acid Stock - dissolve 50 mg standard into methanol using a 50 ml volumetric flask to yield a concentration of 1.0 mg/ml.

7.1.4 Phenoxybenzoic acid Stock - dissolve 50 mg standard into methanol using a 50 ml volumetric flask to yield a concentraion of 1.0 mg/ml.

Spiking Solution:

7.1.5 A 10 ug/ml spike solution was prepared by adding 1.0 ml of each of the above stock solutions to a 100 ml volumetric flask and adjusting the volume to 100 ml with acetonitrile.

GC Calibration Solutions:

7.1.6 A 10 ug/ml mixture of both analytes was prepared by adding 0.5 ml of Fluvalinate stock (7.1.1) and 0.5 ml of haloaniline stock (7.1.2) into 50 ml of toluene using a 50 ml volumetric flask.

7.1.7 GC calibration solutions were prepared in toluene by diluting appropriate volumes of the 10 ug/ml solution (7.1.6) into 50 ml toluene to achieve the following concentrations of both analytes:

7.1.7.1 0.20 ug/ml fluvalinate and haloaniline
7.1.7.2 0.10 ug/ml fluvalinate and haloaniline
7.1.7.3 0.050 ug/ml fluvalinate and haloaniline
7.1.7.4 0.020 ug/ml fluvalinate and haloaniline
7.1.7.5 0.010 ug/ml fluvalinate and haloaniline

7.1.8 HPLC calibration solutions were prepared in mobile phase (7.3) by diluting appropriate volumes of each stock solution (7.1.3 and 7.1.4) into 100 ml of mobile phase to achieve the following concentrations of both acids:

7.1.8.1 1.0 ug/ml CAA and PBA
7.1.8.2 0.50 ug/ml CAA and PBA
7.1.8.3 0.25 ug/ml CAA and PBA
7.1.8.4 0.10 ug/ml CAA and PBA
7.1.8.5 0.050 ug/ml CAA and PBA

7.2 Sample Extraction

7.2.1 Soil Extraction

A 50 gram portion of soil was weighed using a Mettler PE 160 top loading balance and transferred to an 8 oz polyethylene bottle. Following the addition of 200 ml of methanol, the bottle was capped and shaken for 30 minutes on a platform shaker. After settling, the liquid was decanted and 200 ml of 1:1 methanol:water was added to the bottle. The container was shaken another 30 minutes on the shaker. The combined liquid extracts were centrifuged at 3000 RPM for 5 minutes to separate particulates. A 200 ml aliquot was removed from the container for extraction of the analytes.

7.2.2 Partition I (Fluvalinate and Haloaniline)

The 200 ml aliquot was transferred into a 1000 ml Teflon separatory funnel containing 200 ml of 1% sodium bicarbonate. Methylene chloride was added (150 ml) and the funnel was shaken vigorously for one minute. After phase separation, the methylene chloride layer (bottom) was filtered through anhydrous sodium sulfate using a filter funnel and Teflon wool. The organic extract was collected into a 500 ml flat bottom flask. The extraction was repeated using 100 ml of methylene chloride. The aqueous phase was retained for extraction of the acid metabolites, CAA and PBA (Section 7.2.3).

One ml of toluene was added to the combined dried methylene chloride extracts. The solvent was roto-evaporated to approximately 1 ml at 40 - 65 ° C. The residue was quantitatively transferred with small rinses of toluene and adjusted to a final volume of 5 to 10 ml with a 15 ml graduated centrifuge tube using toluene. The toluene extract was stored at 4 +/- 2 ° C prior to GC analysis.

7.2.3 Partition II (Chloroanilino acid and Phenoxy-benzoic acid)

The aqueous phase remaining in the 1000 ml teflon separatory funnel was transferred to a 500 ml flat bottom flask and adjusted to a pH > 11 with 2.0 ml of 9N KOH. The flask was heated for 15 minutes at 65 ° C. The flask was allowed to cool to room temperature and adjusted to pH < 1 using 10 ml of 6N HCL. The acid metabolites were extracted into 50 ml of methylene chloride using a 500 ml Teflon separatory funnel. The organic extract was dried over sodium sulfate and Teflon wool and collected in a 500 ml flat bottom flask. The extraction was repeated using another 50 mls of methylene chloride. The combined dried extracts were roto-evaporated at 65 ° C to approximately 5 mls. The concentrate was quantitatively transferred to a 50 ml centrifuge tube using several volumes of 1:1 methanol:ethyl ether, producing an approximate total volume of 25 mls. The extract was concentrated to < 1 ml on the roto-evaporator. The final volume was adjusted to 2.0 ml using methanol. The extract was stored at 4 +/-2 ° C in an HPLC vial prior to analysis.

7.3 Preparation of HPLC Mobile Phase

A buffer solution of 1.1 M phosphoric acid and 1.0 M sodium acetate was prepared by adding 75 ml of concentrated phosphoric acid and 135 grams of sodium acetate to a 1 liter volumetric flask containing about 500 ml of HPLC grade water. The flask was diluted to the mark using HPLC water. The buffer solution was diluted to 30 mM (30 ml diluted to 1 liter) prior to making a 1:1:1 solution with acetonitrile and methanol as the second and third components. The final concentration of the buffer in the mobile phase was 11 mM for phosphate and 10 mM for acetate.

7.4 Quantitation

Standard calibration curves for were generated using the responses of standards prepared in 7.1.7 and 7.1.8 analyzed intermittently during an analysis sequence. Log/log calibration curves were used for fluvalinate and haloaniline. Linear calibration curves were used for the acid metabolites (responses [peak height] versus the amount injected [ng] were plotted). The curves were used to interpolate sample concentrations using the peak response.

8.0 CALCULATION

8.1 Residue concentrations of each analyte in soil samples were calculated using the following equation.

$$\text{ng/mg (ppm)} = \frac{(\text{ng Found})(\text{Ve})(\text{Vf})(\text{DF})(1000\ \text{ul/ml})}{(\text{Va})(\text{Ws})(\text{Vi})(1000\ \text{mg/g})}$$

where,

ng Found = analyte measured from calibration curve, ng
Ve = volume of initial extract, ml
Vf = volume of final extract, ml
Va = volume of aliquot, ml
Vi = volume injected, ul
DF = dilution factor
Ws = weight of sample, grams

8.2 The limit of detection for fluvalinate and haloaniline was calculated as follows:

$$\text{ng/mg (ppm)} = \frac{(0.020\ \text{ng})(400\ \text{ml})(5.0\ \text{ml})(1)(1000\ \text{ul/ml})}{(200\ \text{ml})(50\ \text{g})(1\ \text{ul})(1000\ \text{mg/g})}$$

$$= 0.004\ \text{ng/mg (ug/g or ppm)}$$

8.3 The limit of detection for CAA and PBA was calculated as follows:

$$\text{ng/mg (ppm)} = \frac{(1.25\ \text{ng})(400\ \text{ml})(2.0\ \text{ml})(2)(1000\ \text{ul/ml})}{(200\text{ml})(50\ \text{g})(25\ \text{ul})(1000\ \text{mg/g})}$$

$$= 0.008\ \text{ng/mg (ug/g or ppm)}$$