1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM060.08A is suitable for the determination of Flumetralin (CGA41065) (Figure 1) in soil. The limit of quantification (LOQ) of the method has been established at 0.01 mg/kg (or 0.01 ppm, 10 ppb).

This method satisfies US EPA guideline OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

Additional clarification added to Section 8.3 in regards to LOQ.
Additional footnotes added to Tables 1&2.

1.2 Method Summary

10 g sub samples of soil are extracted with methanol: water (80/20 v/v). An aliquot of the extract is diluted with aqueous sodium chloride and then partitioned into hexane: toluene (50/50 v/v). Final determination is by GC-NICI-MSD.

The limit of quantification of the method is 0.01 mg/kg (0.01 ppm, 10 ppb).

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare individual 100 µg/mL stock solutions for flumetralin (CGA41065) by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient flumetralin (CGA41065) analytical standard to give a 100 µg/mL solution (≥10 mg, after correction for purity) and transfer into a "Class A" volumetric flask (100 mL). Dilute with an appropriate volume of acetone to give a stock solution of exactly 100 µg/mL of flumetralin (CGA41065). Transfer the standard into an amber bottle for storage.

Alternatively, the appropriate volume of acetone to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P(%)/100)

V = Volume of acetone required

W = Weight, in mg, of the solid analytical standard

C = Desired concentration of the final solution, (µg/mL)

1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solution containing flumetralin (CGA41065) should be prepared by serial dilution in acetone. It is recommended that the following solutions are prepared: 10.0 µg/mL, 1.0 µg/mL and 0.1 µg/mL.

2.3.3 Preparation of Calibration Standards for GC-MSD

No significant suppression or enhancement of the instrument response for flumetralin (CGA41065) has been observed in the soil types tested using the procedures described in Section 3 during method validation and non-matrix standards should normally be used for calibration.

A calibration curve should be generated to quantify flumetralin (CGA41065) residues. At least 5 standards ranging from 0.25 pg/µL to 10.0 pg/µL flumetralin (CGA41065) should be

prepared in hexane: toluene (50/50 v/v). Recommended concentrations: 0.25 pg/µL, 0.5 pg/µL, 1.0 pg/µL, 2.5 pg/µL, 5.0 pg/µL, and 10.0 pg/µL

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in a refrigerator when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of one month for flumetralin (CGA41065) in acetone is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent Hazards

	Toluene	Hexane	Acetone	Methanol
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	✓	✓	✓
Harmful by Skin Absorption	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓
OES Short Term (mg/m^3)	384	N/A	3620	333
OES Long Term (mg/m^3)	191	75	1210	266

N/A not known

Suitable personal protective equipment should be worn when handling chemicals and reagents. The appropriate SDS should be consulted for each reagent and a local risk assessment should be carried out. In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form in Appendix 4.

3.1 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.2 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included with each sample set. To each pre-weighed control soil sample, fortify using flumetralin (CGA41065) in acetone using volumes less than 1 mL. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analysed with each sample set.

3.3 Extraction

a) Weigh a representative amount of soil (10 g) into a 150 mL polypropylene bottle.

b) Add 100 mL methanol: water (80/20 v/v).

c) Place on a mechanical shaker and shake at 275 rpm or at a speed that visually agitates sample for 2 hours.

d) Centrifuge at 3500 rpm for 5 minutes.

3.4 Liquid-liquid Partition

a) Transfer 15 mL of the extract into a polypropylene centrifuge tube (50 mL).

b) Add 15 mL aqueous saturated sodium chloride and 5 mL hexane: toluene (50/50 v/v).

c) Cap and place on a mechanical shaker and shake at 275 rpm for 10 minutes.

d) Centrifuge at 3500 rpm for 5 minutes.

e) Transfer 1.0 mL of the organic layer (upper) into a clean polypropylene centrifuge tube (15 mL).

f) Dilute to 4 mL with hexane: toluene (50/50 v/v).

g) Further dilutions using hexane: toluene (50/50 v/v) can performed at this point if instrument sensitivity permits.

h) Transfer final fraction to a suitable autosampler vial. Final determination is by GC-NICI-MSD. The final sample concentration is 0.075 g/mL.

3.5 Time Required for Analysis

The methodology is normally performed with a batch of 12 samples. One person can complete the analysis of 12 samples in 1 day (8 hour working period).

3.6 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

The method has been developed for use on an Agilent 7890B GC with 5977B MSD. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

4.1 Instrument Description

GC	:	Agilent 7890B
Detector	:	Agilent 5977B
Autosampler	:	Agilent 7693

4.2 Chromatography Conditions

Column	:	HP-5MS (30.0m x 0.25mm x 0.25µm)
Injection Port	:	GooseNeck Carbofrit liner (Restek 20799-209.5)
Carrier Gas	:	Helium at 1.0 mL/min
Injection Mode	:	Pulsed Splitless (pressure 30 psi)
Purge Time	:	1 minutes
Injection Volume	:	2 µL
Injector Temperature	:	250°C
Transfer Line Temperature	:	280°C
Ion Source Temperature	:	150°C
Quadrupole Temperature	:	150°C

Oven Temperature Gradient

Step	Rate (°C/min)	Temperature	Time (min)
1	-	120	1
1	20	300	2

Under these conditions the retention time for flumetralin (CGA41065) is approximately 9.3 minutes.

4.3 Mass Spectrometer Conditions

Ionization Mode : Chemical (NICI)

Polarity : Negative

Calibration : AutoTune

Analyte : Flumetralin (CGA41065)

Target Ion : 421 *m/z*

Qualifier 1 : 423 *m/z*

Qualifier 2 : 391 *m/z*

Ion Ratio : 100:70:20

4.4 Confirmatory Procedures

Final determination by GC-MS with two qualifier ions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

Flumetralin (CGA41065) residues may be calculated in mg/kg for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (30% LOQ to at least 20% above the highest fortified level as a minimum). An appropriate number of different concentrations within this range should be prepared (at least five).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to flumetralin (CGA41065). Calibration standard solutions should be interspersed throughout the analysis, bracketing the first and last samples analysed in the run.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for *x* gives

$$x = \frac{y - c}{m}$$

e) Calculate the flumetralin (CGA41065) residue in the sample, expressed as mg/kg, as follows

$$\text{Residue (mg/kg)} = \frac{\text{Analyte found } (\mu\text{g/mL})}{\text{Sample conc. (g/mL)}}$$

Where analyte found (μg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in g/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

5.2 Single-Point Calibration Procedure

Flumetralin (CGA41065) residues may be calculated in mg/kg for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing flumetralin (CGA41065) at an appropriate concentration into the GC-MSD operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for flumetralin (CGA41065).

b) Make an injection of each sample solution and measure the areas of the peak corresponding to flumetralin (CGA41065).

c) Re-inject the standard solution after a maximum of five injections of sample solutions.

d) Calculate the flumetralin (CGA41065) residue in the sample, expressed as mg/kg using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue (mg/kg)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (μg/mL)
Sample Conc. = Sample concentration (g/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 3).

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analysed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with known amounts of flumetralin (CGA41065) in acetone) should also be analysed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The fortification levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq$20%.

Where the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix Effect

No significant interference arising from the matrices tested has been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

APPENDIX 3 GC-MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis. Perform instrument auto tune of compound specific tune using specific calibration masses.

Tuning Instrument for flumetralin

Determine ionization mode and detection (EI or CI).

Perform scan of expected masses. Determine target ion and qualifier ions. Target plus two qualifiers above 100 amu are recommended.

For flumetralin, in negative ion chemical ionization mode, the deprotonated molecular ion generated is selected (*m/z* 421) as the target ion. The two most sensitive qualifier ions (*m/z* 423 and *m/z* 391) are then selected for confirmation.

Daughter Ion *m/z*	Structure
423	^{37}Cl isotope
391	Loss of H_2O

APPENDIX 4 Method Flow Chart