1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM060.09A is suitable for the determination of flumetralin (CGA41065) (Figure 1) in water. The limit of quantitation (LOQ) of the method has been established at 0.05 μg/L (or 0.05 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

Surface water samples (15 mL) are partitioned into hexane: toluene (50/50 v/v) and analyzed directly by GC-NICI-MSD.

The limit of quantitation of the method is 0.05 μg/L (0.05 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 a 100 μg/mL stock solution for flumetralin by one of the following methods:

Note: the amount weighed out must be corrected for the purity of the analytical standard as indicated on the certificate of analysis and also any salt content, where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.

Weigh out accurately, using a five figure balance, sufficient flumetralin analytical standard into separate amber "Class A" volumetric flasks (100 mL size). Dilute to the mark with acetone to yield an individual 100 μg/mL stock solutions of flumetralin.

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 and any salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.

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

P	=	Standard purity (including correction for salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.) 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 solutions containing flumetralin (CGA41065) should be prepared by serial dilution in acetone from the stock solution. It is recommended that the following solutions are prepared: 1.0 μg/mL, 0.10 μg/mL and 0.01 μg/mL for fortification purposes.

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 water 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 0.05 pg/µL to 10.0 pg/µL flumetralin (CGA41065) should be prepared in hexane: toluene (50/50 v/v). Recommended concentrations: 0.05 pg/µL, 0.10 pg/µL, 0.5 pg/µL, 1.0 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
Harmful Vapor	✓	✓	✓
Highly Flammable	✓	✓	✓
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	✖	✖	✖
OES Short Term (mg/m^3)	384	N/A	3620
OES Long Term (mg/m^3)	191	75	1210

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

If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity 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 water sample, fortify using flumetralin (CGA41065) in acetone using volumes less than 1 mL. At least one untreated control and two fortified control samples should be analyzed with each sample set.

3.3 Extraction

a) Transfer 15 mL of the water sample to be analyzed into a 50 mL polypropylene centrifuge tube

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 an aliquot from the organic layer (upper) into a suitable autosampler vial for final determination by GC-NICI-MSD. The final sample concentration is 0.15 µg/L. Further dilution can be performed using hexane: toluene (50/50 v/v) if instrument sensitivity permits.

3.4 Experimental Precautions

To prevent contamination of the instrument and to minimize possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 0.1 mg/kg should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL. It may also be useful to include solvent blank injections after high level samples to clear any observed carry-over greater than 10% of the LOQ.

3.5 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples. One skilled analyst can complete the analysis of 1-2 sample sets in 1 day (8 hour working period).

3.6 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends 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 optimization 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 (SIM)

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 for Flumetralin (CGA41065)

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

Residues of flumetralin (CGA41065) may be calculated in µg/L 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 analyzed 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 (slope) 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 residues of interest in a sample, expressed as µg/L, as follows:

$$Residue\ (\mu g/L\ or\ ppb) = \frac{Analyte\ Found\ (pg)}{Water\ Sample\ Injected\ (mg\ or\ \mu L)}$$

Where on-column *Analyte Found (pg)* is calculated from the standard calibration curve and on-column *Water Sample (matrix) Injected* is calculated as follows:

$$Water\ Sample\ Injected\ (mg\ or\ \mu L) = Sample\ Volume\ (mL) \times \frac{Injection\ Volume\ (\mu L)}{Sample\ Final\ Volume\ (mL)}$$

f) Determine the recovery by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery as a percentage (%) by the equation:

$$Recovery\ (\%) = \frac{(Residue\ in\ Recovery\ Sample) - (Residue\ in\ Control)}{Amount\ Fortified} \times 100\%$$

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

$$Corrected\ Residue\ (\mu g/L\ or\ ppb) = \frac{Residue\ (\mu g/L\ or\ ppb)}{Average\ Precent\ Recovery}$$

5.2 Single Point Calibration Procedure

Flumetralin (CGA41065) residues may be calculated in µg/L (ppb) 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 peaks corresponding to flumetralin (CGA41065).

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

d) Calculate the flumetralin (CGA41065) residues in the sample, expressed as µg/L (ppb) using a mean standard response from each of the injections bracketing the sample as follows:

$$Residue\ (\mu g/L\ or\ ppb) = \frac{PK\ area\ (SA)}{PK\ area\ (STD)} \times \frac{Standard\ Conc.}{Sample\ Conc.}$$

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 (L/mL)

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

$$Corrected\ Residue\ (\mu g/L\ or\ ppb) = \frac{Residue\ (\mu g/L\ or\ ppb)}{Average\ Precent\ Recovery}$$

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 2).

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed 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 analyzed with each batch of samples. Control samples from the same matrix are recommended to monitor any instrumental matrix effects present.

At least two recovery samples (control samples accurately fortified with known amounts of analyte), including one at the method LOQ and one at the expected residue level, should also be analyzed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found in the sample. The fortification levels should be appropriate to the residue levels expected in the sample.

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

When 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

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 for GC-MSD

Transfer sample (15 mL) to a 50 mL polypropylene tube

↓

Fortify recovery sample, if needed

↓

Perform LLP with salt using 15 mL extract and 5 mL toluene: hexane (50:50 v/v)

↓

Centrifuge (3500 rpm)

↓

Transfer 1 mL supernatant (organic phase) to autosampler vial and analyse by GC-NICI-MSD