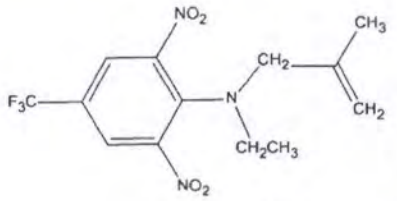
INTRODUCTION

Ethalfluralin is a member of the cell division inhibitor class of herbicides. The mode of action is that of a selective soil herbicide, which acts as an inhibitor of various physiological growth processes, the structure is shown below.



Ethalfluralin
CAS number 55283-68-6

Common name, chemical name and structure along with the nominal mass are given in the Certificate of Analysis (Appendix A).

This report contains method validation data for a Dow AgroSciences residue analytical method for the determination of ethalfluralin in drinking water, ground water and surface water, for the intent of updating the ChemService residue analytical method number 19/2002, "Determination of Ethalfluralin Residues in Drinking, Ground and Surface waters by Gas Chromatography with Electron Capture Detection" (Reference 1) to meet a lower limit of quantification of 5.0 ng/L and to meet current SANCO guidelines (SANCO 825/00 rev. 8.1, Reference 2).

The final extracts were analyzed for residues of ethalfluralin using gas chromatography with electron-impact mass spectrometry detection (GC/MS).

The purpose of this study was to provide validation data to define the accuracy, precision, specificity and ruggedness of this method and to fulfill data requirements outlined in the U.S. EPA Residue Chemistry Test Guidelines, OCSPP 850.6100 (Reference 4) and SANCO/825/00 rev.8.1 (Reference 2).

The analytical procedure was demonstrated to be applicable for use in the determination of ethalfluralin in water. The limit of quantitation was confirmed to be 5.0 ng/L.

ANALYTICAL

Storage of Samples

The analytical method validation was carried out on three water specimens: surface water, ground water and drinking water. The drinking water was obtained from a "drinking water" tap at the Test Facility (CEMAS), the ground water was obtained from a well near Henley-on-Thames and the surface water was obtained from The Cut River, Bracknell, UK.

Specimen	CEMAS Sample Reference Number
Surface Water	CCON/037/004
Ground Water	CCON/038/004
Drinking Water	CCON/039/002

On receipt, the specimens were stored at approximately 4 °C before and after analysis.

GLP Characterisation of Samples

The water specimens were characterised at CEMAS, Study Number CEMS-5748. Details of the GLP characterisation results are as follows:

Specimen	pH	Total Hardness (mg/L as $CaCO_3$)	Total Suspended Solids (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Dissolved Organic Carbon (mg/L)
Surface Water	7.5	185.2	22.0	108.0	5.5
Ground Water	7.3	196.2	3.1	265.0	0.2
Drinking Water	7.6	280.3	2.5	204.0	5.4

Specimen	Electrical Conductivity (µS/cm)	Silt Content (mg/L)	Bicarbonate (mg/L)	Carbonate (mg/L)
Surface Water	697	45	131.8	<0.1
Ground Water	584	55	323.3	<0.1
Drinking Water	693	<1	248.9	<0.1

pH of water specimens

The pH of the water samples was determined using CEMAS SOP CEM-3373 - Determination of the pH of Water, Soil and Sediment Samples in Water and/or Salt Solutions (0.01 M Calcium Chloride, 0.1 M Potassium Chloride, 1.0 M Potassium Chloride).

The pH value reflects the relative number of hydrogen ions (H^+) in solution. The more hydrogen ions present, compared to the hydroxyl ions (OH^-), the more acidic the solution will be and the lower the pH value. A decrease in hydrogen ions and increase in hydroxyl ions will result in more alkaline or basic conditions.

The pH was determined, potentiometrically, using a glass combination electrode and a pH meter.

Hardness EDTA titration

Total Hardness by EDTA Titration in water was determined using CEMAS SOP CEM-3060 – Determination of Total Hardness by EDTA Titration in Water.

Water hardness is an expression for the sum of the calcium and magnesium cation concentrations in a water sample. The standard method of expressing water hardness is in mg/L calcium carbonate ($CaCO_3$) which has the formula weight of 100.1 g/mole.

Water hardness was determined using a complexometric titration method using a standard ethylenediaminetetraacetic acid (EDTA) solution. Due to steric hindrances, EDTA will complex with calcium and magnesium in a one-to-one molar ratio. Since EDTA and its hardness complexes are not colored, an additional chelating agent, eriochrome black T, was used to facilitate endpoint detection.

Total Suspended Solids

The total suspended solids in the water samples were determined using CEMAS SOP CEM-3448 – Determination of Total Suspended and Volatile Suspended Solids in Waters, which is a standard gravimetric procedure. Total suspended solids are described as those solids which are retained on a glass fibre filter and dried at 103-105°C. Five hundred mL of sample was filtered, under vacuum, onto a pre-weighed glass fibre filter (GF/F). The paper plus residue was dried for at least two hours and then reweighed. The weight of residue was expressed as mg/L total suspended solids.

Carbonate, Bicarbonate, Carbonate Hardness and Alkalinity

Alkalinity was determined using CEMAS SOP CEM-3384 - Determination of Alkalinity of Water – Carbonate, Bicarbonate and Carbonate Hardness.

Alkalinity is the measure of a water sample's ability to neutralize hydrogen ions (its acid-neutralizing ability). Alkalinity may be caused by dissolved strong bases such as sodium hydroxide or potassium hydroxide (and other hydroxide-containing compounds), and it may, also, be caused by dissolved carbonates, bicarbonates, borates, and phosphates. The measured alkalinity is the total of all of these species found in a water sample. For the sake of simplicity, alkalinity was expressed in terms of mg $CaCO_3$/L although many species other than dissolved calcium carbonate may actually contribute to the alkalinity. Total Alkalinity is referred to as Carbonate Hardness.

The carbonate concentration was determined by titration with hydrochloric acid using phenolpthalein as an indicator and the bicarbonate hardness level was determined by further titration with the same acid using bromophenol blue as the indicator.

Silt Content

The silt content of water was measured using CEMAS SOP CEM-3385 - Determination of particle size distribution in Water, Fractionation/sedimentation Method.

The sand fraction was removed from the water specimen by sieving. The silt and clay particles after suspension were sampled with a pipette at different sedimentation times and were then determined gravimetrically. The method is dependent on the fact that the sedimentation rate of particles in water is proportional to their size and temperature of the water (Stokes Law).

Dissolved and Total Organic Carbon

The dissolved organic carbon and the total organic carbon of the water samples were determined using CEMAS SOP CEM-3396 - Determination of the Total and Dissolved Organic Carbon, Inorganic Carbon and Carbon in Water. The dissolved organic carbon (DOC) is a measure of the organic material, contained in a water sample that is soluble and/or colloidal, that can pass through a 0.45µm filter.

A Sievers Model 5310C TOC Analyser (GE Analytical Instruments) was used to measure the concentration of Dissolved Organic Carbon (DOC), Inorganic Carbon (DIC), and Carbon (DTC) in the water samples. The analyser principle is based on the oxidation of organic compounds to form carbon dioxide using UV radiation and a chemical oxidising agent (ammonium persulphate). Carbon dioxide is measured using a sensitive, selective membrane-based conductometric detection technique. For each TOC measurement, the concentration of inorganic carbon species (carbonates, bicarbonates and carbon dioxide) is determined and, after oxidation of the organic compounds, the total carbon (TC) content of the sample is measured. The concentration of the organic compounds (TOC) was calculated from the difference between the concentrations of the dissolved carbon (DTC) and inorganic carbon (DIC). As the water samples were filtered, results were expressed as dissolved organic carbon (DOC).

Reagents and Consumables

Methanol, HPLC grade, catalog number M/4056/17, Fisher Scientific.

Ethyl acetate, HPLC grade, catalog number A/3446/50, Fisher Scientific.

Water, HPLC grade, catalog number W/0106/17, Fisher Scientific.

Strata C18 SPE Cartridges (500mg, 6mL), Part no. 8B-S002-HCH, Phenomenex.

Preparation of Standards

The following analytical test substances/analytical standards were utilized during the method validation:

Test Substance/ Analytical Standard:	Ethalfluralin
Supplier:	Dow AgroSciences (Sponsor)
Test Substance No.	TSN101281
Batch/Lot no:	597-C049-003
Purity:	99.8%
Expiry date:	08 January 2013
Storage:	Coldroom

Preparation of Ethalfluralin Spiking Solutions

A 0.020 g portion of ethalfluralin analytical standard was weighed and quantitatively transferred to a 20-mL volumetric flask with methanol. The standard was diluted to volume with methanol to obtain a 1000-µg/mL stock solution. Further dilutions of the 1000-µg/mL spiking solution with methanol were performed as follows:

Concentration of Initial Stock Solution	Aliquot of Stock Solution	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]	Volume of Spiking Soln.
µg/mL	mL	mL	µg/mL	ng/L	mL
1000	0.5	100	5.0	- -	- -
5.0	0.5	50	0.05	50	0.50
0.05	5.0	50	0.005	5	0.50
- -	- -	- -	0.005	1.5	0.15

[a] The equivalent sample concentration is based on fortifying a 500-mL water sample.

Preparation of Ethalfluralin Calibration Solutions

A 0.020 g portion of ethalfluralin analytical standard was weighed and quantitatively transferred to a 20-mL volumetric flask with methanol. The standard was diluted to volume with methanol to obtain a 1000-µg/mL stock solution. Dilutions of the 1000-µg/mL stock solution with methanol were performed to obtain 10-µg/ml and 1.0-µg/ml calibration standard solutions.

Further dilution of the 1.0-µg/mL stock solution with ethyl acetate was performed to give calibration standards over the range 0.75–100 ng/mL. Calibration standards were prepared using the following scheme:

Concentration of Stock Solution	Aliquot of Stock Solution	Final Soln. Volume	Calibration Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	ng/mL	ng/L
1.0	5.0	50	100.0	200
1.0	2.5	50	50.0	100
1.0	1.0	50	20.0	40
0.1	5.0	50	10.0	20
0.1	2.5	50	5.0	10
0.1	1.0	50	2.0	4
0.01	7.5	100	0.75	1.5

[a] The equivalent sample concentration is based on fortifying a 500-mL water sample.

Fortification of Recovery Samples

The control specimens were fortified as described below:

Matrix	Untreated Control Specimens	Replicates at Fortification Level (LOD)*	Replicates at Fortification Level (LOQ)**	Replicates at Fortification Level
Drinking water	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L
Ground water	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L
Surface water	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L

*LOD – Limit of determination
**LOQ – Limit of quantitation

Five-hundred-mL aliquots of the control specimen were measured into individual graduated cylinders. Each sample was fortified as per the table above. One sample was fortified to achieve the fortification level 1.5 ng/L (LOD), five samples were fortified at 5.0 ng/L (LOQ) and five samples were fortified to achieve the upper fortification levels of 50.0 ng/L. The fortification solution was injected directly into the matrix.

Sample Extraction, Purification and Analysis

Samples were purified using a C-18 solid-phase extraction (SPE) column. After elution from the SPE column with 6-mL of ethyl acetate, the ethyl acetate was evaporated from the final eluate (some water may be present at this stage) and dissolved in 1 mL ethyl acetate. Final samples were vortex mixed and analyzed by gas chromatography with electron-impact mass spectrometry detection (GC/MS).

Full extraction details:

Sample Analysis

1. Measure 500 mL portions of each matrix into individual graduated cylinders and then add 4 mL of methanol to each sample. For recovery samples, add an appropriate volume of the spiking solution to obtain concentrations ranging from 1.5 to 50.0 ng/L for all the water matrices.
2. Manually mix samples and leave to stand for 5 minutes.
3 Purify samples on 500-mg C-18 SPE columns on a vacuum manifold.
    a. Place SPE columns on the manifold.
    b. Condition the SPE column with 4 mL of ethyl acetate, followed by 4 mL of methanol and 4 mL of ultrapure water. Do not apply vacuum to the SPE manifold at this stage.
    c. Place a 100 ml empty reservoir to the top of each cartridge with a suitable adapter.
    d. Transfer the sample was to the reservoir. Draw the sample through the column at 4-5 ml/min flow rate by application of vacuum, discard the eluate. Apply vacuum at the end to drain the sample completely.
    e. Elute Ethalfluralin from the SPE column with 6 ml of ethyl acetate, do not apply vacuum initially, and then apply vacuum at the end to drain the sample completely.

4. Evaporate samples using a stream of nitrogen with a Techne Dri-block concentrator until all ethyl acetate is removed (some water may be present at this stage), dissolve samples in 1mL ethyl acetate.

5 Transfer samples and calibration standards to auto-sampler vials (taking care not to transfer any water present). Analyze by GC/MS.

Equipment, glassware, materials, reagents and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests.

Analytical Instrumentation and Equipment

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.

Instrumentation:	Agilent Model 6890A gas chromatograph
	Agilent Model 7683 autoinjector
	Agilent Model 5973N mass spectrometer
	Agilent Model G1701CA data system
Column	J & W fused silica capillary
	Durabond-5MS liquid phase
	30 m x 0.25 mm i.d. 0.25-µm film thickness
Liner:	p/n: 5183-3316
Oven :	100 °C for 1.5 min
	to 140 °C at 5 °C/min
	to 150 °C at 1.0 °C/min, hold for 3.0 min
	to 280 °C at 50 °C/min, hold for 5.0 min
Transfer Line	280 °C

Carrier Gas method	Helium
Constant Flow	1.4 ml/min
Linear Velocity	~44 cm/s

Injection Method:	Splitless
Temperature:	250 °C
Pressure:	14.98psi
Purge flow:	50.0ml/min
Purge time:	0.90 min
Total flow:	54.2 mL/min

Electron Impact with selected ion monitoring (EI-mode)

Source Temperature	230 °C
Quad Temperature:	150 °C
Electron Multiplier	1900 volts (~200 volts above autotune)
SIM Resolution	Low
Dwell Time	300 msec

Monitored:

m/z 276 (quantitation)
m/z 292 (confirmation 1)
m/z 316 (confirmation 2)

Calculation of Results

For each analytical batch, a range of calibration standards was injected over the range 0.75 ng/mL to 100 ng/mL for ethalfluralin. A calibration curve was prepared by plotting the quantitation peak area obtained versus analyte concentration using lineal regression forced through zero (the type of regression model can be chosen to give the best fit for the data).

Example

Ethalfluralin recovery at 5.0 ng/L in drinking water

ASR number = 1196/12/04

Peak area ethalfluralin = 10311

Slope of calibration curve (forced through origin) = 4366.913

$$\text{Ethalfluralin concentration in final extract} = \frac{Peak\ area\ ethalfluralin}{slope}$$

$$\text{Ethalfluralin concentration in final extract} = \frac{10311}{4366.913} = 2.361164\ \text{ng/mL}$$

Concentration = 0.5 L/mL
Dilution factor = 1

$$\text{Ethalfluralin residue} = \frac{Ethalfluralin\ conc.\ in\ final\ extract\ \times Dilution\ factor}{Concentration}$$

$$\text{Ethalfluralin residue} = \frac{2.361164\ \times 1}{0.5} = 4.722\ \text{ng/L}$$

Mean residue in control sample = 0.0000 ng/L, used to subtract any background contributions

$$\text{Recovery} = \frac{Ethalfluralin\ residue - mean\ residue\ in\ control\ sample}{Fortification\ level} \times 100$$

$$\text{Recovery} = \frac{4.722 - 0.0000}{5.0} \times 100 = 94\%$$

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level of one matrix type was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Confirmation of Residue Identity

Confirmation was performed to demonstrate the selectivity of the primary method by monitoring two additional ions simultaneously with the primary detection method which used one ion in GC-MS. The analyte signal of the primary method is considered to be quantitatively correct and not affected by any other compound when the retention time of the sample matches that of the standards, and the confirmation ratio for the sample is in the range of ±20% of the average confirmation ratio found for the standards. Untreated control matrix samples and samples fortified at the lowest fortification level for each analyte/matrix combination are provided to prove selectivity of the method.