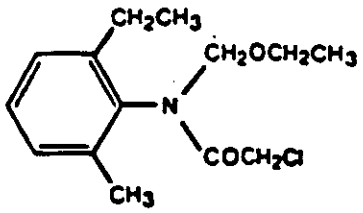
1. SCOPE

The analytical procedure described is suitable for the determination of 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide (I) (Acetochlor, ICIA5676) in soil using external standardisation. The limit of determination of the method is 0.01 mg kg^{-1}

(I)



Acetochlor

2. METHOD SUMMARY

A prepared soil sample is extracted by shaking with acetonitrile/water (1:1 v/v) for 30 minutes at room temperature. Vacuum filtration is performed and the aqueous extract then partitioned with dichloromethane. A silica bond elut column clean up may be used to reduce interfering soil coextractives before determination by gas chromatography (G.C.) using a nitrogen selective thermionic detector.

3. PROCEDURE

3.1 Sample Preparation

Soil samples received in zero contamination tubes should be allowed to thaw for approximately half to one hour prior to preparation.

The soil from the plastic tubes is either bulked or divided into the various depths required for analysis. The bulked sample is weighed and put through a 2 mm sieve to remove any stones, roots etc before the sample is reweighed. The soil is then thoroughly mixed before taking a representative sample for analysis.

A moisture content should be evaluated on the sample. (A known weight of soil (approximately 20g) is placed in a petri dish and dried to constant weight by placing in an oven at 120°C maximum).

3.2 **Extraction**

a) Weigh representative samples of soil in duplicate (20g) into round-bottom flasks and spike at least two recovery samples with standard (dissolved in acetonitrile) as required. (See Section 6). Two untreated control samples, one reagent blank and one spiked reagent blank should also be included. (See Section 5).

b) Add Acetonitrile : Water (1:1) (50ml) to the round-bottom flasks and stopper - using adhesive tape to retain stoppers firmly. Shake the samples on a mechanical shaker for 30 minutes, at room temperature.

c) Filter the extracts under vacuum through Whatman filter paper No. 5 and wash the retained solids with 2 x 20ml of acetonitrile.

3.3 **Partition**

a) Transfer the filtrates to separating funnels (250ml) containing water (50ml) and dichloromethane (100ml). Shake for 1 minute and leave to separate.

b) Run the lower organic layer off into a round-bottom flask and rotary evaporate to dryness.

c) Dissolve the residue in Hexane (10ml) to give a 2 g ml^{-1} sample solution ready for GC injection.

3.4 **Silica Bond Elut™ Column Clean-up (optional)**

A silica clean-up is only necessary if the sample is required to be more concentrated or if interferences are present in the GC determination.

1) Pre wet Silica Bond Elut™ column with Hexane (2ml).
2) Put soil extract onto column (2ml of solution in 3.3(c) = 4g soil).
3) Wash through with Hexane (2ml) and then discard the wash.
4) Either -

a) Elut Acetochlor fraction using Hexane:Acetone (4:1) (2ml) and collect it. This gives a sample concentration of 2 g ml^{-1} as in 3.3 (c).

or

b i) Wash through with Hexane:Acetone (4:1) (1ml) and discard the wash.

ii) Elut acetochlor with Hexane:Acetone (4:1) (1ml) collecting the fraction for analysis by G.C. (This gives a sample concentration of 4 g ml^{-1}.)

The bond elut column must not dry out at any stage during this procedure.

Note : Prior to use, each fresh lot no of Bond Elut™ columns should be calibrated as follows:-

Take a control sample (20 g), spiked at a level of 0.5 mg kg^{-1} (ie 1.0 ml of a 10 g ml^{-1} standard acetochlor solution in acetonitrile) through the extraction and partition procedures described in sections 3.2 and 3.3.

When carrying out the clean-up procedure in section 3.4 the Bond Elut columns are calibrated by collecting the pre-wash and eluate in 1 ml fractions for individual analysis by GC.

The acetochlor elution pattern is therefore determined and may be used to check that all the analyte occurs in the expected fraction (ie section 3.4.4 a or b).

3.5 Gas/Liquid Chromatography

The following conditions gave satisfactory results using a Hewlet Packard HP5890A chromatograph with a Nitrogen selective thermionic detector.

3.5.1 Chromatographic conditions

Column and column packing	: Silicone SE52/4 (immob) Capillary column 25 m x 0.32 mm id x 0.5 m film thickness
Carrier gas - Helium	: 4.2 ml min^{-1}
Make up gas - Helium	: 33.3 ml min^{-1}
Hydrogen	: 3.4 ml min^{-1}
Air	: 96.8 ml min^{-1}
Chart Speed	: 0.5 cm min^{-1}
Column temperature	: 50°C/1mins 20°C/min 250°C/10mins
Detector temperature	: 300°C
Injector temperature	: 220°C
Injection volume	: 1 ul

Acetochlor has a retention time of 10.04 minutes under these conditions in these laboratories.

Typical chromatograms are shown in Appendix 5.

3.5.2 Calculation of Results

i) Acetochlor residues may be calculated in $mgkg^{-1}$ for each sample extract using a mean standard response from injections bracketing the sample as follows:

$$\text{Residue} = \frac{\text{Pk height (Sample)}}{\text{Pk height (Std)}} \times \frac{\text{W (Std)}}{\text{I (Sample)}} \times \frac{\text{V(s)}}{\text{W(s)}} \; mgkg^{-1}.$$

Where
- Pk height (Sample) = Peak height or area for sample/cm
- Pk height (Std) = Average peak height or area for bracketing standards/cm
- W (Std) = Weight of acetochlor in reference standard/ng
- I (Sample) = Sample solution injection volume/ml
- V (s) = Solvent volume in sample extract/ml
- W (s) = Sample weight equivalent to sample extract/g

ii) These sample residues should be further corrected using the average percentage recovery. (i.e. calculate each recovery sample as above, and express as a percentage of the fortification level. Then average all the recovery percentages for use in the calculation below)

$$\text{Corrected Residue } mg/kg^{-1} = \frac{\text{Residue} \times 100}{\text{Average percentage recovery}}$$

iii) Results should finally be corrected for the soil moisture content using the following equation:

$$\text{Dry weight residue } /mgkg^{-1} = \frac{\text{Wet Weight Residue} \times 100}{100 - \% \text{ Soil Moisure Content}}$$

Results should be corrected to two significant figures (S.F.) or one S.F. if the residue is <0.1 $mgkg^{-1}$

4. LIMIT OF DETERMINATION

A true assessment of the limit of determination of the method may be determined by fortification of untreated samples at low levels with acetochlor and subjecting them to the complete analytical procedure. The chromatographic response obtained for these recoveries at the retention time of 10.04 minutes, should exceed the background signal noise by a factor of at least four to be considered an acceptable quantitative limit of determination. In addition the precision of measurement at this level should not exceed a coefficient of variation of $\pm$ 15%.

In these laboratories the limit of determination has been set at 0.01 mg kg^{-1}.

5 REAGENT BLANKS/CONTROLS

The analyst must verify that the sample chromatograms are free from interferences orginating from either untreated soil samples or from the reagents, and that sample contamination with acetochlor has not occurred before or during the analysis. Therefore at least two control crop samples and one reagent blank (whole procedure followed in absence of soil) should be analysed along side each set of samples. It is also useful to take through a spiked reagent blank to indicate whether anomalous results are due to the method, or adsorption/binding of the analyte by the crop, and to check that the spiking standard solution agrees with the standard in hexane used for GC analyses.

6 RECOVERIES

A minimum of two external recovery experiments should be run alongside each set of samples analysed; (that is untreated samples accurately fortified with a known amount of acetochlor prior to extraction), in the range 0.05 - 0.5 mg kg^{-1}.

For external standard recoveries, fortification levels should be based on the expected soil residue levels. When no residues are expected the recoveries should be fortified at low levels typically 0.1 mg kg^{-1} and include at least one fortified at the limit of determination. Provided the recovery values obtained are acceptable (mean values >85%; confidence limit $\pm$ 15%) they may be used to correct the acetochlor residue as in Section 3.5.2.

7 METHOD VALIDATION

a) Calibration graph for Standards and Recoveries.

A series of recoveries were fortified at acetochlor levels between 0.5 mg kg^{-1} and 0.01 mg kg^{-1}. These were then taken through the method. A series of standards in hexane were also run and graphs plotted of peak height/mm versus acetochlor concentration 1/ug ml^{-1} (See Appendix 6).

Linear relationships were observed for the standards and the recoveries were very close to the standard line.

APPENDIX 1 : Apparatus

a) Equipment for the initial preparation of samples e.g. a Hobart Band Saw for cutting soil cores.

b) Rotoshake mechanical shaker supplied by R W Jennings & Co Ltd Nottingham, UK.

c) Vacuum rotary evaporator with thermostatically controlled water bath, available from Buchi, Switzerland.

d) Complete VAC ELUT™ ten place vacuum manifold assembly with sample collector rack (for use with BOND ELUT™ disposable extraction columns). Jones Chromatography Ltd UK.

e) Gas liquid chromatograph fitted with a nitrogen selective thermionic detector, e.g. Hewlet Packard 5890A GC with an autosampler, HP7673.

f) 1 mV recorder, e.g. Kipp & Zonen BD40.

g) Silicone SE52/4 (immob) capillary column 25 m x 0.32 mm id x 0.5 um film thickness from Thames Chromatography, 16 Raymead Court, Maidenhead, Berkshire SL6 8TN.

APPENDIX 2 : Reagents

a) Solvents: glass distilled grade acetonitrile, dichloromethane, hexane, acetone.

b) Ultra pure water

c) BOND ELUT™ disposable extraction columns (2.8 ml) containing 500 mg unbonded silica. Supplier:- Jones Chromatography Ltd, UK (Part No 601303, manufactured by Analytichem International Inc, USA).

d) 2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide (acetochlor, ICIA5676) (Known purity, >96%)

APPENDIX 3 : 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 doubt, consult the appropriate safety manual (e.g. ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory' edited by L Bretherick, The Royal Society of Chemistry, London.

a) DICHLOROMETHANE

Harmful vapour
Irritating to eyes
Avoid breathing vapour
Avoid contact with eyes and skin
(CL 350 mg m^{-3})

b) ACETONITRILE

Toxic by inhalation, in contact with skin
Highly flammable
Do not breath vapour
Avoid contact with eyes and skin
(RL 70 mg m^{-3})

c) HEXANE

Highly flammable
Harmful by inhalation and in contact with skin
Possible risk of irreversible effects
Avoid breathing vapour
Avoid contact with skin and eyes
(RL 360 mg m^{-3})

d) ACETONE

Highly flammable
Irritating to eyes
Avoid breathing vapour
Prevent contact with eyes
(CL 2400 mg m^{-3})

e) 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide (acetochlor, ICIA5676)

Avoid contact with eyes, skin and clothing.
Avoid breathing vapours

APPENDIX 4 : Preparation of Analytical Standards

It is recommended that the following handling precautions should be taken when weighing the analytical standard material.

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

Weigh out accurately, using a five figure balance, sufficient 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide to allow dilution in acetonitrile to give a 1000 ug ml^{-1} stock solution in volumetric flask. Make serial dilutions of this stock solution to give 100 ug ml^{-1}, 10 ug ml^{-1} and 1.0 ug ml^{-1} standard solutions in acetonitrile. These solutions should be used for the fortification of recovery samples.

Also prepare a dilution of 1 ug ml^{-1} standard solution in Hexane for use in the gas chromatographic analysis.

When not in use, standard solutions should always be stored in a refrigerator at 4°C to prevent evaporation and concentration. Analytical standards should be replaced with freshly prepared standards after four months of use.