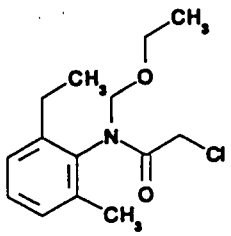
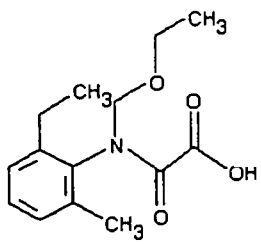
1 SCOPE

The analytical method described is suitable for the determination of residues of the herbicide acetochlor and its oxanilic acid metabolite in various water types. The limit of quantification (LOQ) for acetochlor is 0.05 $\mu g\ l^{-1}$ and for acetochlor oxanilic acid is 1.0 $\mu g\ l^{-1}$.

Structure of Acetochlor
2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide



Structure of Acetochlor Oxanilic Acid
N-ethoxymethyl-N-(2-ethyl-6-methylphenyl)oxamic acid



2 METHOD SUMMARY

In summary, water is passed through a C_{18} solid phase extraction (SPE) column, retaining the analytes on the column. Acetochlor is eluted using hexane : dichloromethane (DCM). Residue determination is by gas-liquid chromatography (GC) with mass selective detection (MSD). Acetochlor oxanilic acid is eluted from the SPE column using methanol and reduced to dryness. Following reconstitution in acetonitrile, N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) is added to form the tertiary butyl dimethylsilyl derivative. Residue determination is by GC/MSD.

3 PROCEDURE

3.1 Sample Collection

Ground water or lysimeter water samples are collected from source using sampling techniques consistent with those employed for low level residue analysis. Ideally samples should be collected directly into high density polyethylene (HDPE) bottles (500 ml volume or greater).

3.2 Sample Work-up

(a) Accurately measure out 250 ml (± 2 ml) of water sample into a 250 ml measuring cylinder. Transfer the water to a suitable container e.g. storage jar.

NB. If 250 ml of water is not available, take the maximum volume possible (>50 ml) through the analytical procedure. In this case the LOQ for both analytes will need to be reevaluated.

(b) Fortify at least two control samples by the addition of an appropriate amount (1 ml or less) of an acetochlor standard in methanol and an oxanilic acid standard in acetonitrile, using either a syringe or pipette.

(c) An untreated control sample must be analysed routinely with each set of samples. A reagent blank should also be analysed with each set of samples.

(d) The samples may then be acidified (at the discretion of the Study Director or Principal Investigator - see note below) by adding concentrated hydrochloric acid (HCl) (2 ml) to each. If less than 250 ml sample volume has been taken, adjust the volume of acid accordingly eg. add 0.8 ml of HCl to 100 ml of water.

NB. For certain water types (specifically some ground water samples), it has been noted that acidification of the sample causes a stronger interaction of acid metabolites with the matrix, resulting in the analytes being strongly retained on the SPE column. As a result, all the metabolite is not eluted from the column and low recoveries are observed. If this phenomenon is encountered, the method should be repeated with the acidification step removed.

3.3 Solid Phase Extraction (SPE) Sample Preparation

Before this clean-up procedure is first used, the columns should be calibrated using the procedures detailed in Appendix 3 Section 5.

3.3.1 Sample Application

(a) Prepare the solid phase clean-up apparatus by inserting the relevant number of C_{18} endcapped columns (1 g) into the adaptors on top of a vacuum manifold. Connect the manifold to a vacuum line.

(b) Condition each column using the following procedure. Pipette methanol (5 ml) on to the top of each column and apply the vacuum. Draw the solvent through the column at a rate of ~5 ml min^{-1} down to the column frit to prevent the column going dry. Repeat the procedure using ultra-pure water (5 ml).

(c) Certain water types either visibly contain a degree of sediment or by experience take an excessive time (> 1 hour) to elute through the SPE column. If this is observed fit into each reservoir (60 ml) two 20 µm frits to prevent blockage of the SPE column.

(d) Fix a column adaptor into the top of each column and insert a reservoir (with or without frits). Load the sample into the reservoir and draw the water through the column under a medium vacuum (flow rate of ~ 20 ml min^{-1}). Once all the water has been loaded, switch to maximum vacuum for approximately 10 minutes to dry the columns.

NB. Excessive column drying (>30 minutes) may result in the loss of acetochlor.

Alternatively, the water may be transferred onto the column under vacuum through plastic tubing. Pipette ultra-pure water (4 ml) onto the top of each column. Insert a length of plastic tubing of the correct diameter through the hole in the column adaptor to obtain a seal. Place the other end of the tube into the water container and apply the vacuum, thus drawing the water through the column.

(d) Close the vacuum and if any globules of water are retained on the walls of the C_{18} column absorb them onto absorbant laboratory paper roll. Remove the adaptors and reservoirs and place suitable labelled collection vessels in the rack within the vacuum manifold.

3.3.2 Elution of Parent Acetochlor

(a) Pipette hexane/dichloromethane (40:60 v/v)(6 ml) or as determined from the column calibration (Appendix 3, Section 5) onto the top of each column. Reapply a low vacuum and elute the parent acetochlor into the collection tubes at a rate of ~5 ml min^{-1}, again only drawing the solvent through to the level of the column frit.

In certain circumstances, a small volume of water may have been retained on the column and will be eluted into the collection tubes. This will be clearly visible as the lower immiscible layer. Remove the aqueous layer using a Pasteur pipette and continue with the method.

(b) Dilute all samples to an accurate volume (5 ml) using hexane/dichloromethane (40:60 v/v) and transfer an aliquot (~1.5 ml) to an appropriate autosampler vial in preparation for analysis.

NB. If a volume of water <250 ml was available to extract, the final sample volume may be decreased in order to lower the LOQ. In this case the sample will be evaporated to a known volume (eg. 1 ml) under a steady flow of air.

3.3.3 Elution of Acetochlor Oxanilic Acid

(a) Place suitable labelled collection vessels in the rack in the vacuum manifold eg. screw cap vials with Teflon lined caps (7 ml).

(b) Pipette methanol (5 ml) or as determined from the column calibration (Appendix 3, Section 5) onto the top of each column. Reapply a low vacuum to elute the acetochlor oxanilic acid into the collection tubes at a rate of ~5 ml min^{-1}, again only drawing the solvent through to the level of the column frit.

(c) Pipette further methanol (2 ml) onto the top of each column. Reapply a low vacuum to elute the acetochlor oxanilic acid into the collection tubes at a rate of ~5 ml min^{-1} and draw the solvent through the columns to dryness. The columns will have thus been eluted with a total of 7 ml methanol.

(d) Reduce the methanol to dryness under a steady stream of dry air. Accurately reconstitute each sample in acetonitrile (0.8 ml) and ultrasonicate thoroughly (approximately 30 seconds per sample). Shake the samples during ultrasonication to ensure complete uptake of any material adhering to the sides of the tubes.

3.4 **Preparation of Tertiary Butyl Dimethylsilyl Derivative of Acetochlor Oxanilic Acid**

(a) Prepare a standard solution in duplicate for GC/MSD comparison at a level similar to expected residues. If no residues are expected, a 0.25 µg ml^{-1} standard should be prepared (this is equivalent to a 1.0 µg l^{-1} residue). In an equivalent vessel to 3.3.3(a), take a 1 µg ml^{-1} acetochlor oxanilic acid standard (0.1 ml) and add acetonitrile (0.7 ml). Recap the vials and ultrasonicate thoroughly (approximately 30 seconds per sample).

NB. If a different standard concentration is being prepared, the final volume should always be 1 ml.

(b) Add MTBSTFA (0.2 ml) to each sample and standards, replace the caps and ultrasonicate thoroughly (approximately 30 seconds per sample). Shake the samples during ultrasonication to ensure complete uptake of any material adhering to the sides of the tubes.

(c) Heat the samples and standards on a heating block at 100°C for 1 hour, agitating occasionally. Remove the vials after one hour and allow to cool at room temperature.

(d) Transfer the samples and standards to an appropriate autosampler vial in preparation for analysis.

3.5 Gas-liquid Chromatography

Analysis should be carried out using a gas-liquid chromatograph (GC) fitted with a mass selective detector (MSD). A standard solution must be injected after a maximum of four sample injections. If particularly dirty traces are obtained with certain water types, a washing solvent (eg. acetone) should be injected at intervals throughout the analytical run.

A small amount of carry over may be observed following the injection of higher level oxanilic acid tertiary butyldimethylsilyl derivative standard solutions. If this is observed and is of a significant level to effect low level sample traces, a reagent blank or control should routinely be run after standard injections.

The conditions for the analysis by GC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe optimum use. The low level work described in this text should ideally be carried out on a 'clean system' to ensure optimum results. This will require a new column, septum, liner and clean injection port prior to the initial work commencing. Running this method alongside other environmental analyses may result in poor chromatography and loss of sensitivity.

The following conditions have been found to be satisfactory in our laboratories using the instruments detailed below.

3.5.1 Acetochlor Determination

3.5.1.1 Gas-liquid Chromatograph with Mass Selective Detection (GC/MSD)

Gas-liquid chromatograph	:	HP5890 series 2
Detector	:	HP5972 MSD or equivalent
Autosampler	:	HP7673A

3.5.1.2 Gas-liquid Chromatograph Conditions

Column	:	HP5 (30 m x 0.25 mm id x 25 µm df)
Injection technique	:	Splitless
Head Pressure	:	8.3 psi
Injection Volume	:	1 µl

(NB. this may be increased to a maximum of 5 µl using these conditions)

Liner	:	4 mm id Restek presilanised double gooseneck packed with a plug of silanised pesticide grade glass wool.
Injector Temperature	:	250°C
Detector Temperature	:	275°C
Temperature Program	:	60°C(1 min) to 240°C @ 20°C min^{-1} to 280°C(2 min) @ 40°C min^{-1}

(NB. During the analysis of certain matrix types containing more organic coextractants, it may be necessary to modify the temperature program in order to obtain acceptable chromatograms eg. lower the initial ramp to 10°C min^{-1})

3.5.1.3 Mass Selective Detector Conditions

Acquisition Mode	:	Selective Ion Monitoring (SIM)
Solvent Delay	:	9.50 min
Electron Multiplier	:	2500 volts
Electron Energy	:	70 eV
System Calibration	:	Manual tunes carried out weekly using ions 131,219,219.

NB. This factor is critical in achieving required sensitivity.

Compound Groups	:	Group 1 Acetochlor
Target Ions	:	m/z 146, 162
Dwell time per ion	:	150 msec
Resolution	:	Low
Group Start Time	:	9.50 min

3.5.1.4 Detector Linearity

Before this method is used to determine residue levels in water samples, a range of acetochlor standards (comparable to expected residue levels) should be injected using the conditions above to check the linearity of the detector. If the response is not linear over the required range, a residue which falls outside the linear range must either be accurately diluted until it falls within the linear range or run against an equivalent strength standard.

3.5.2 Acetochlor Oxanilic Acid Determination

3.5.2.1 Gas-liquid Chromatograph with Mass Selective Detection (GC/MSD)

Gas-liquid chromatograph	:	HP5890 series 2
Detector	:	HP5972 MSD or equivalent
Autosampler	:	HP7673A

3.5.2.2 Gas Liquid Chromatograph Conditions

Column	:	Rtx-200 (20 m x 0.18 mm id x 0.2 µm df)
Injection technique	:	Splitless
Head Pressure	:	5 psi
Injection Volume	:	1 µl

(NB. this may be increased to a maximum of 5 µl using these conditions)

Liner	:	4 mm id restek presilanised double gooseneck packed with a plug of silanised pesticide grade glass wool.
Injector Temperature	:	250°C
Detector Temperature	:	275°C
Temperature Program	:	60°C (1 min) to 170°C (5 min) @ 10°C min^{-1} to 280°C (1.33 min) @ 30°C min^{-1}

3.5.2.3 Mass Selective Detector Conditions

Acquisition Mode	:	Selective Ion Monitoring (SIM)
Solvent Delay	:	15.4 min
Electron Multiplier	:	2750 volts
Electron Energy	:	70 eV
System Calibration	:	Manual tunes carried out weekly using ions 131, 219, 219.
Compound Groups	:	Group 1 Acetochlor Oxanilic Acid
Target Ions	:	m/z 146, 175
Dwell time per ion	:	150 msec
Resolution	:	Low
Group Start Time	:	15.40 min

3.5.2.4 Detector Linearity

Before this method is used to determine residue levels in water samples, a range of acetochlor oxanilic acid standards (comparable to expected residue levels) should be injected using the above conditions to check detector linearity. If the response is not linear over the required range, residues which fall outside the linear range must be accurately diluted until they fall within the linear range or run against an equivalent strength standard.

3.6 **Calculation of Results**

Residues of both analytes may be calculated in $\mu g\ l^{-1}$ for each sample extract using a mean response signal from the standard injections bracketing that sample as follows:

$$\text{Residue} = \frac{\text{Res (SA)}}{\text{Res (STD)}} \times \frac{\text{Conc (STD)}}{\text{Conc (SA)}} \times 1000 \times \frac{\text{Inj (STD)}}{\text{Inj (SA)}}$$

Res(SA) = Peak height / area for sample
Res(STD) = Average peak height / area for bracketing calibration standards
Conc(STD) = Concentration of compound in standard ($\mu g\ ml^{-1}$)
Conc(SA) = Concentration factor of water in final sample (ie. 250 ml to 5 ml = 50)
Inj(STD) = Standard injection volume (μl)
Inj(SA) = Sample injection volume (μl)

These sample residues should not be further corrected for average percentage recovery unless specifically required. If residues need to be corrected (eg. storage stability work) then the equation below should be used:

$$\text{Corrected Residue} = \frac{\text{Residue}}{\text{APR}} \times 100\ \mu g / 1$$

APR = Average Percentage Recovery. Never correct sample residues down where the APR is greater than 100%.

Acetochlor and acetochlor oxanilic acid results should be routinely calculated using ion m/z = 146.

Ratios between ion m/z = 146 and ion m/z = 162 for acetochlor should be consistent between samples and standards. In these laboratories the ratio is approximately 1.4. Samples showing contamination at the acetochlor retention time of ion m/z = 146 may be calculated using ion m/z = 162 at the discretion of the analyst.

Ratios between ion m/z = 146 and ion m/z = 175 for the acetochlor oxanilic acid t-butyldimethylsilyl derivative should be consistent between samples and standards. In these laboratories the ratio is approximately 1.3. Samples showing contamination at the acetochlor oxanilic acid t-butyldimethylsilyl derivative retention time of ion m/z = 146 may be calculated using ion m/z = 175 at the discretion of the analyst.

4 RECOVERIES

A minimum of two external recoveries must be analysed alongside each set of samples analysed.

Fortification levels should be based on the expected residue levels, but at least one of the recoveries should be fortified at twice the limit of quantification (LOQ) (ie. 0.10 $\mu g\ l^{-1}$ for acetochlor and 2.0 $\mu g\ l^{-1}$ for acetochlor oxanilic acid).

Recovery values obtained should generally be between 70 - 120 % with a %CV within a run of not greater than 15, for that run to be acceptable. Variations outside these parameters should only be accepted at the Study Directors' or Principal Investigators' discretion.

5 LIMIT OF QUANTIFICATION (LOQ) AND LIMIT OF DETERMINATION (LOD)

The LOQ of this method is set at 0.05 $\mu g\ l^{-1}$ for acetochlor and 1.0 $\mu g\ l^{-1}$ for acetochlor oxanilic acid. It is defined as the lowest level at which the analytical method has been successfully validated.

Care must be taken to ensure that the LOQ is always greater than the LOD. The LOD is defined as the lowest level at which an analyte can be determined with a reasonable degree of statistical certainty above instrumental background noise, which for the purpose of this method should be a signal equivalent to at least four times the mean amplitude of the background signal.

6 REAGENT BLANKS/CONTROLS

At least one control sample and one reagent blank should be analysed alongside each set of samples.

7 METHOD VALIDATION SUMMARY

7.1 Method Accuracy and Precision

In these laboratories to date, the method described above has been applied to the analysis of suction lysimeter water and ground water, but should be applicable to all water types. A range of accurately fortified untreated samples of each commodity were taken through the analytical procedure and calculated against external standards. The range of recovery values obtained are presented in Tables 1 and 2.

1. Apparatus

a) Measuring cylinders (250 ml) available from VWR Scientific, PO Box 7900, San Francisco, CA 94120 (Tel: 415-467-6202).

b) Polypropylene wide neck storage bottles (250 ml), available from VWR Scientific, PO Box 7900, San Francisco, CA 94120 (Tel: 415-467-6202).

c) Solid Phase Extraction Columns (1 g C_{18} endcapped), available from Varian Sample Preparation Products, 24201 Frampton Avenue, Harbor City, CA 90710 (Tel: 310-539-6490).

d) 75 ml frits (20 µm), available from Varian Sample Preparation Products, 24201 Frampton Avenue, Harbor City, CA 90710 (Tel: 310-539-6490).

e) Vacuum Manifold, available from Varian Sample Preparation Products, 24201 Frampton Avenue, Harbor City, CA 90710 (Tel: 310-539-6490).

f) Graduated centrifuge tubes (Precalibrated) available from VWR Scientific, PO Box 7900, San Francisco, CA 94120 (Tel: 415-467-6202).

g) Screw cap vials with Teflon lined caps (7 ml), available from Supelco Inc. Supelco Park, Bellefonte, PA 16823-0048 (Tel: 814-359-3441)

h) Heating module (to 150°C) with aluminium blocks for close fit of vials, available from Pierce, PO Box 117, Rockford, IL 61105 (Tel: 815-968-0747).

i) Gas chromatograph with a Mass Selective Detector, eg. HP5890 and autosampler HP7673A plus detector (HP5972 MSD) and standalone PC integrator. Available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: 800-223-9700).

j) Analytical gas chromatography capillary columns :
30 m x 0.25 mm id with a 0.25 µm df: HP 5 from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: 800-223-9700).

k) Crimp cap autosampler vials, microvials and caps, available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: 800-223-9700).

2. Reagents

a) Concentrated hydrochloric acid, available from Curtin Matheson Scientific Inc., General Offices: 9999, Veterans Memorial Drive, Houston, TX (Tel: 713-820-9898).

b) Solvents: Methanol, acetonitrile, hexane and dichoromethane (high purity solvents), available from Curtin Matheson Scientific Inc., General Offices: 9999, Veterans Memorial Drive, Houston, TX (Tel: 713-820-9898).

c) Ultra pure water as produced by Elga Ultra Pure Still Maxime or equivalent. Available from Elga Inc., 430 Old Boston Road, Topsfield, MA 01983 (Tel: 508-887-6300).

d) N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), available from Pierce, PO Box 117, Rockford, IL 61105 (Tel: 815-968-0747).

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 any doubt, consult the appropriate safety manual (eg. ZENECA Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by L Bretherick, The Chemical Society, London.

a) Solvent Hazards.

	Methanol	Acetonitrile	Hexane	Dichloromethane
Harmful Vapour	Y	Y	Y	Y
Highly Flammable	Y	Y	Y	N
Risk of Irreversible Effects	N	N	Y	Y
Recommended Limit (RL) /ppm	200	40	100	100

In all cases avoid breathing vapour. Avoid contact with skin and eyes.

b) ZENECA Agrochemicals Toxicity Classifications.

Acetochlor, dichloromethane and MTBSTFA have a divisional toxicity class of 2 (highly toxic).

4 Preparation of Analytical Standards

4.1 Acetochlor Standards

Weigh out accurately using a five figure balance, sufficient analytical standard to allow dilution in methanol to give a 1000 $\mu g\ ml^{-1}$ stock solution in a volumetric flask. Make serial dilutions of this standard to give 100 $\mu g\ ml^{-1}$, 10 $\mu g\ ml^{-1}$, 1.0 $\mu g\ ml^{-1}$ and 0.1 $\mu g\ ml^{-1}$ in methanol. These standards are used to fortify samples.

Standard solutions in hexane should also be prepared to produce a 0.01 $\mu g\ ml^{-1}$ standard in dichlormethane : hexane (60:40 v/v) to routinely use as a bracketing standard when quantifying samples. The hexane standards should be diluted from the 100 $\mu g\ ml^{-1}$ standard solution in methanol, to give 10 $\mu g\ ml^{-1}$, 1.0 $\mu g\ ml^{-1}$, 0.1 $\mu g\ ml^{-1}$ and 0.01 $\mu g\ ml^{-1}$ standards in hexane.

Due to the fact that methanol and hexane are immiscible, it is necessary to evaporate the methanol just to dryness prior to diluting with hexane. THE STANDARD MUST NOT BE LEFT AT DRYNESS DUE TO VOLATILITY OF THE COMPOUND.

Finally, prepare a 0.01 $\mu g\ ml^{-1}$ standard in dichloromethane : hexane (60:40 v/v) by dilution of the 0.1 $\mu g\ ml^{-1}$ standard in hexane.

When not in use, always store the standards solutions in a refrigerator at <7°C to prevent decomposition and/or concentration of solvent strength. Analytical standards are assumed to be stable for 4 months from the date of the preparation of the initial stock solution.

After this period, a new set of standard solutions must be prepared. These should be checked according to Standard Operating Procedure 41/083/--.

4.2 Acetochlor Oxanilic Acid Standards

Weigh out accurately using a five figure balance, sufficient analytical standard to allow dilution in acetonitrile to give a 1000 $\mu g\ ml^{-1}$ stock solution in a volumetric flask. Make serial dilutions of this standard to give 100 $\mu g\ ml^{-1}$, 10 $\mu g\ ml^{-1}$ and 1.0 $\mu g\ ml^{-1}$ standards in acetonitrile.

When not in use, always store the standards solutions in a refrigerator at <7°C to prevent decomposition and/or concentration of solvent strength. Analytical standards are assumed to be stable for 4 months from the date of the preparation of the initial stock solution.

After this period, a new set of standard solutions must be prepared. These should be checked according to Standard Operating Procedure 41/083/--.

5 **SPE Column Calibration**

The following method is the recommended procedure for calibrating the SPE columns.

(a) Prepare the solid phase clean-up apparatus by inserting the relevant number of C_{18} endcapped columns (1 g, 6 cc) into the adaptors on top of a vacuum manifold. Connect the manifold to a vacuum line.

(b) Condition each column using the following procedure. Pipette methanol (5 ml) onto the top of each column and apply the vacuum. Draw the solvent through the column at a rate of ~5 ml min^{-1} down to the column frit to prevent the column going dry. Repeat the procedure using ultra-pure water (5 ml).

(c) Fortify ultrapure water (250 ml) with 0.25 µg of acetochlor (equivalent to a 1.0 µg l^{-1} recovery) and 2.5 µg of acetochlor oxanilic acid (equivalent to a 10 µg l^{-1} recovery) in duplicate. Add HCl (2 ml) to each sample.

(d) Fix a column adaptor into the top of each column and insert a reservoir (60 ml). Load the sample into the reservoir and draw the water through the column under a medium vacuum (flow rate of ~ 20 ml min^{-1}). Once all the water has been loaded, maintain maximum vacuum for approximately 10 minutes to dry the columns.

(e) Close the vacuum and if any globules of water are retained on the walls of the C_{18} column absorb them onto absorbant laboratory paper roll. Remove the adaptors and reservoirs and place suitable labelled collection vessels in the rack within the vacuum manifold.

(f) Pipette hexane/dichloromethane (40:60 v/v) (6 ml) onto the top of each column. Reapply a low vacuum and elute the analyte into the collection tubes at a rate of ~5 ml min^{-1}, again only drawing the solvent through to the level of the column frit.

(g) Dilute each sample to an accurate volume (eg. 5 ml) using hexane/dichloromethane (40:60 v/v) and transfer an aliquot (~1.5 ml) to an appropriate autosampler vial and analyse using the conditions described in section 3.5.1.

(h) If 95% or more of the acetochlor has been recovered, the columns are calibrated and the method is ready for use. However if all the acetochlor is not accounted for, repeat the above procedure increasing the composition of dichloromethane in the elution solvent in steps of 20% until 95% or more of the acetochlor is accounted for.

(i) Place suitable labelled collection vessels in the rack in the vacuum manifold eg. screw cap vials with Teflon lined caps (7 ml).

(j) Pipette methanol (5 ml) onto the top of each column. Reapply a low vacuum to elute the acetochlor oxanilic acid into the collection tubes at a rate of ~5 ml min^{-1}, again only drawing the solvent through to the level of the column frit. Repeat the procedure with a further aliquot of methanol (2 ml).

(k) Reduce the methanol to dryness under a steady stream of dry air. Accurately reconstitute each sample in acetonitrile (0.8 ml) and ultrasonicate thoroughly. Follow the procedures described in section 3.4.

(l) If 90% or more of the acetochlor oxanilic acid has been recovered, the columns are calibrated and the method is ready for use. However if all the acetochlor oxanilic acid is not accounted for, elute the columns with further fractions of methanol (1 ml) until 90% or more of the acetochlor oxanilic acid has been recovered.