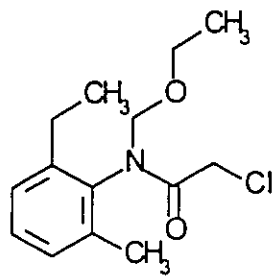
1 SCOPE

The analytical method described is suitable for the determination of residues of the herbicide acetochlor and its sulphonic acid metabolite in various water types. The limit of quantification (LOQ) for acetochlor is 0.05 µg L^{-1} and for acetochlor sulphonic acid is 1.0 µg L^{-1}.

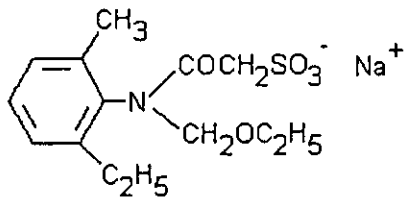
Structure of Acetochlor

2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide



Structure of Acetochlor Sulphonic Acid (as the sodium salt)

N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-3-oxoethane sulphonic acid



2 METHOD SUMMARY

In summary, water is passed through a C_{18} solid phase extraction (SPE) column on which the analytes are retained. Acetochlor is eluted using dichloromethane (DCM) : hexane. Residue determination is by gas-liquid chromatography (GC) with mass selective detection (MSD).

The acetochlor sulphonic acid fraction is eluted from the C_{18} SPE column with methanol onto a SAX SPE column. The SAX column is washed with acetonitrile : ultra pure water followed by ammonium acetate : methanol : acetonitrile. Finally the acetochlor sulphonic acid is eluted from the SAX column using ammonium acetate : methanol : acetonitrile. Residue determination is by high performance liquid chromatography (HPLC) with ultra-violet (UV) detection.

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, the stated LOQ for each analyte may not be achievable. In these laboratories, the LOQ for acetochlor and for acetochlor sulphonic acid can be achieved with a minimum volume of 50 ml and 150 ml respectively.

(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 acetochlor sulphonic 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) Add glacial acetic acid (0.75 ml) to each sample. If less than 250 ml sample volume has been taken, adjust the volume of acid accordingly eg. add 0.3 ml of acetic acid 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 these clean-up procedures are first used, the columns should be calibrated using the procedures detailed in Appendix 3 Section 5.

3.3.1 Application

(a) Prepare the solid phase extraction apparatus by inserting the relevant number of C_{18} endcapped columns (1 g, 6 cc) into the adaptors on top of a vacuum manifold and 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}). When the total volume of 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.

or

The water may be transferred onto the column using a Zymark AutoTrace SPE workstation. The workstation is operated according to the procedures described in the operator's manual. In summary, column conditioning, sample loading, column drying and analyte elution steps can be automated by using the workstation.

(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.

3.3.2 Elution of Parent Acetochlor

(a) Pipette dichloromethane : hexane (60 : 40 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 parent acetochlor into the collection tubes at a rate of ~5 ml min^{-1}, again only draw the solvent through to the level of the column frit.

NB. 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 a 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 dichlormethane : hexane (60:40 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 is available to extract, the final sample volume may be decreased to retain an equivalent LOQ. In this case, place the sample under a steady stream of dry air and reduce the volume to a known amount (eg. 1 ml). **DO NOT** reduce the sample to dryness, as this will result in the loss of acetochlor.

3.3.3 Ion Exchange Sample Preparation and Elution of Acetochlor Sulphonic Acid

(a) Insert the relevant number of SAX SPE columns (500 mg, 3 cc) into the adaptors on top of a vacuum manifold. Condition each column using the following procedure : pipette methanol (2.5 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 with a further aliquot of methanol (2.5 ml).

(b) Using an adaptor, insert the C_{18} columns from 3.3.2 (a) on top of the conditioned SAX columns.

(c) Pipette methanol (5 ml) [or as determined from the column calibration (Appendix 3, Section 5)] onto the top of each C_{18} column. Reapply a low vacuum and elute the solvent through the C_{18} and SAX columns at a rate of ~5 ml min^{-1} down to the column frit on the SAX column. Repeat the procedure with a further aliquot of methanol (2 ml). Remove and discard the C_{18} columns.

(d) Wash each SAX column using the following procedure : Pipette ultra pure water acetonitrile (50 : 50 v/v) (2.5 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. Repeat the procedure with a further aliquot of ultra pure water : acetonitrile (50 : 50 v/v) (2.5 ml).

(e) Pipette 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v) (1 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.

(f) Place suitable labelled collection vessels in the collection rack and elute the SAX columns with 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v) (2.5 ml). Again elute to the top frit of the column at a rate of ~ 5 ml min^{-1}. This gives a final volume of 2.5 ml and a concentration factor of 100 if 250 ml was taken for analysis.

(g) Transfer an aliquot (~1.5 ml) to an appropriate autosampler vial in preparation for analysis with an acetochlor sulphonic acid standard.

3.4 Gas-liquid Chromatography

Acetochlor 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.

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.

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

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

3.4.1.1 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}

3.4.1.2 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

3.4.1.3 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 High Performance Liquid Chromatography (HPLC / LC)

Acetochlor sulphonic acid analysis should be carried out using an LC fitted with a ultra-violet (UV) detector. A standard solution must be injected after a maximum of four sample injections.

The conditions for the analysis by LC 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 and good separation. Running this method alongside other environmental analyses may result in poor chromatography and loss of sensitivity. For optimum separation at the temperature specified below, an average column will last for approximately 150 sample injections after which a new column should be utilised.

The following conditions have been found to be satisfactory in our laboratories:

3.5.1 Liquid Chromatograph Conditions

Column	:	S5ODS2 (25 cm x 3.2 mm id)
Mobile Phase	:	78 : 11 : 11 v/v/v 0.05 M Ammonium Acetate : Methanol : Acetonitrile
Flow	:	0.5 ml min^{-1}
Column Temperature	:	60°C
Detector Wavelength	:	220 nm
Injection Volume	:	100 µl

3.5.2 Detector Linearity

Before this method is used to determine residue levels in water samples, a range of acetochlor sulphonic acid 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.

3.6 **Calculation of Results**

Residues of both analytes may be calculated in µg 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 (µg 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 :

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

$$\text{Corrected Residue} = \frac{\text{Residue}}{\text{APR}} \times 100\ \mu g\ L^{-1}$$

Acetochlor 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.

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 µg L^{-1} for acetochlor and 2.0 µg L^{-1} for acetochlor sulphonic acid).

Mean recovery values obtained should generally be between 70 - 110 % with a % coefficient of variation (CV) within a run of not greater than 20, for that run to be acceptable. Variations outside these parameters should only be accepted at the Study Director's or Principal Investigator's discretion.

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

The LOQ of this method is set at 0.05 µg L^{-1} for acetochlor and 1.0 µg L^{-1} for acetochlor sulphonic acid. The LOQ 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.

The minimum criteria to achieve the LOQ of this method is an analyte signal equivalent to at least 1.5 times the LOD (ie. six times above instrumental background noise), for that peak to be accurately quantified.

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 - 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 (500 mg, 3cc SAX and 1 g, 6cc 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) and reservoirs, 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) Zymark AutoTrace SPE workstation. Available from Zymark Co., Zymark Center, Hopkinton, Massachusetts 01748-9965 (Tel: 508-435-9761).

h) 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).

i) 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).

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

k) High performance liquid chromatograph with an ultra violet detector, eg. HP1100 with column oven and standalone PC integrator. Available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: 800-223-9700).

l) Analytical liquid chromatography columns :
25 cm x 3.2 mm id: S5ODS2 from Phenomenex, 2320 W 205th Street, Torrance, CA 90501.

2. Reagents

a) Glacial acetic 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 dichloromethane (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) Ammonium Acetate (crystals, guaranteed reagent), available from VWR Scientific, PO Box 7900, San Francisco, CA 94120
(Tel: 415-467-6202).

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 and dichloromethane both 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 µg ml^{-1} stock solution in a volumetric flask. Make serial dilutions of this standard to give 100 µg ml^{-1}, 10 µg ml^{-1}, 1.0 µg ml^{-1} and 0.1 µg ml^{-1} in methanol. These standards are used to fortify samples.

Standard solutions in hexane should also be prepared to produce a 0.01 µg ml^{-1} standard in dichloromethane : hexane (60:40 v/v) to routinely use as a bracketing standard when quantifying samples. The hexane standards should be diluted from the 100 µg ml^{-1} standard solution in methanol, to give 10 µg ml^{-1}, 1.0 µg ml^{-1}, 0.1 µg 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 re diluting in hexane. THE STANDARD MUST NOT BE LEFT DUE TO VOLATILITY OF THE COMPOUND.

Finally, prepare a 0.01 µg ml^{-1} standard in dichloromethane : hexane (60:40 v/v) by dilution from the 0.1 µg 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 have been shown 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 Sulphonic Acid Standards

Weigh out accurately using a five figure balance, sufficient analytical standard to allow dilution in acetonitrile : ultra pure water (90 :10 v/v) to give a 1000 µg ml^{-1} stock solution in a volumetric flask. Make serial dilutions of this standard to give 100 µg ml^{-1}, 10 µg ml^{-1} and 1.0 µg ml^{-1} standards in acetonitrile.

Standard solutions in mobile phase should also be prepared to produce a 1.0 µg ml^{-1} standard to routinely use as a bracketing standard when quantifying samples. These should be diluted from the 100 µg ml^{-1} standard solution in acetontrile, to give a 10 µg ml^{-1} and 1.0 µg ml^{-1} standards in 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v).

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 have been shown 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.

5.1 C_{18} Endcapped Column Calibration

(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 ultra pure water (250 ml) with 0.25 µg of acetochlor (equivalent to a 1.0 µg l^{-1} recovery) and with 2.5 µg of acetochlor sulphonic acid (equivalent to a 10 µg l^{-1} recovery) in duplicate. Add glacial acetic acid (0.75 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 dichloromethane : hexane (60:40 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 dichlormethane : hexane (60:40 v/v) and transfer an aliquot (~1.5 ml) to an appropriate autosampler vial and analyse using the conditions described in section 3.4.1.

(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.

(j) Pipette methanol (5 ml) onto the top of each column. Reapply a low vacuum to elute the acetochlor sulphonic 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). The columns will have thus been eluted with a total of 7 ml of methanol.

(k) Reduce the methanol to dryness under a steady stream of dry air. Accurately reconstitute each sample in 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v)(1.0 ml) and ultrasonicate thoroughly. Analyse using the conditions described in section 3.5.1.

(l) If 95% or more of both acetochlor sulphonic acid and acetochlor oxanilic acid has been recovered, the columns are calibrated and the method is ready for use. However if all the acetochlor sulphonic acid and / or acetochlor oxanilic acid is not accounted for, elute the columns with further fractions of methanol (1 ml) until 95% or more of both acids have been recovered.

5.2 **SAX Column Calibration**

(a) Prepare the solid phase clean-up apparatus by inserting the relevant number of SAX columns (500 mg, 3 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 (2.5 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 with a further aliquot of methanol (2.5 ml).

(c) Fortify methanol (7 ml) with 2.5 µg of acetochlor sulphonic acid (equivalent to a 10 µg L^{-1} recovery) in duplicate.

(d) Load the sample onto the column and draw through under a low vacuum (flow rate of ~ 5 ml min^{-1}). Only draw the solvent through to the top frit of the SAX column.

(e) Wash the SAX columns using the following procedure : Pipette ultra pure water : acetonitrile (50 : 50 v/v) (2.5 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. Repeat the procedure with a further aliquot of ultra pure water : acetonitrile (50 : 50 v/v)(2.5 ml).

(f) Wash the SAX columns with 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v) (1.0 ml). Draw the solvent through the column at a rate of ~5 ml min^{-1} down to the column frit.

(g) Place suitable labelled collection vessels in the collection rack and elute the SAX columns with 0.1M ammonium acetate : methanol : acetonitrile (78 : 11 : 11 v/v/v) (2.5 ml). Again elute to the top frit of the column at a rate of~ 5 ml min^{-1} and analyse using the conditions described in section 3.5.1.

(i) If 95% or more of the acetochlor sulphonic acid has been recovered, the columns are calibrated and the method is ready for use. However if all the acetochlor sulphonic acid is not accounted for, elute the columns with further fractions of mobile phase (0.5 ml) until 95% or more of the sulphonic acid has been recovered.